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L.M.B.C. MEMOIRS.

XXIII.

TUBIFEX

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Liverpool Marine Biology Committee.

L.M.B.C. MEMOIRS

ON TYPICAL BRITISH MARINE PLANTS & ANIMALS

EDITED BY W. A. HERDMAN, D.Sc., F.R.S.

XXIII.

TUBIFEX

BY

GERTRUDE C. DIXON, B.Sc.

LECTURER IN BIOLOGY, KING'S COLLEGE FOR WOMEN, LONDON

[THESIS APPROVED FOR THE DEGREE OF DOCTOR OF SCIENCE
IN THE UNIVERSITY OF LONDON]

(With 7 Plates)

Price Three Shillings and Sixpence

LONDON
Williams & Norgate
August, 1915

EDITOR'S PREFACE.

The Liverpool Marine Biology Committee was constituted in 1885, with the object of investigating the Fauna and Flora of the Irish Sea.

The dredging, trawling, and other collecting expeditions organised by the Committee have been carried on intermittently since that time, and a considerable amount of material, both published and unpublished, has been accumulated. Twenty-eight Annual Reports of the Committee and five volumes dealing with the "Fauna and Flora" have been issued. At an early stage of the investigations it became evident that a Biological Station or Laboratory on the sea-shore nearer the usual collecting grounds than Liverpool would be a material assistance in the work. Consequently the Committee, in 1887, established the Puffin Island Biological Station on the North Coast of Anglesey, and later on, in 1892, moved to the more commodious and accessible Station at Port Erin in the centre of the rich collecting grounds of the south end of the Isle of Man. A larger Biological Station and Fish Hatchery, on a more convenient site at Port Erin, has since been erected, and was opened for work in July, 1902.

In these twenty-eight years' experience of a Biological Station, where College students and amateurs form a large proportion of the workers, the want has been frequently felt of a series of detailed descriptions of the structure of certain common typical animals and plants, chosen as representatives of their groups, and dealt with by specialists. The same want has probably been felt in other similar institutions and in many College laboratories.

The objects of the Committee and of the workers at the Biological Station were at first chiefly faunistic and speciographic. The work must necessarily be so when opening up a new district. Some of the workers have published papers on morphological points, or on embryology and observations on life-histories and habits; but the majority of the papers in the volumes on the "Fauna and Flora of Liverpool Bay" have been, as was intended from the first, occupied with the names and characteristics and distribution of the many different kinds of marine plants and animals in our district. And this faunistic work will still go on. It is far from finished, and the Committee hope in the future to add still further to the records of the Fauna and Flora. But the papers in the present series, started in 1899, are quite distinct from these previous publications in name, in treatment, and in purpose. They are called "L.M.B.C. Memoirs," each treats of one type, and they are issued separately as they are ready, and will be obtainable Memoir by Memoir as they appear. It is hoped that such a series of special studies, written by those who are thoroughly familiar with the forms of which they treat, will be found of value by students of Biology in laboratories and in Marine Stations, and will be welcomed by many others working privately at Marine Natural History.

The forms selected are, as far as possible, common L.M.B.C. (Irish Sea) animals and plants of which no adequate account already exists in the text-books. Probably most of the specialists who have taken part in the L.M.B.C. work in the past will prepare accounts of one or more representatives of their groups. The following list shows those who have either performed or promised.

The subject of the present Memoir is usually regarded as a fresh-water animal; but it is frequently found in brackish tidal waters; and, moreover, it is a useful type of aquatic Oligochaeta, so I had no hesitation in including "Tubifex" in the series.

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In addition to these, it is hoped that other Memoirs will be arranged for, on suitable types, such as Pontobdella, a Cestode, a Copepod and a Pycnogonid.

W. A. HERDMAN.

University of Liverpool,
June, 1915.

L.M.B.C. MEMOIRS.

No. XXIII. TUBIFEX

BY

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[*From the Zoological Department, King's College, London.*]

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INTRODUCTION.

I. METHODS.

My investigations have been carried out on the living worm by teasing out certain parts of the body, and by means of a large number of series of transverse and longitudinal sections.

I have found the method of teasing out certain parts of the worm, either in water or salt solution, quite invaluable. By this means all the reproductive organs can be liberated and examined in the living condition, and very pretty staining effects have been obtained by the addition of methylene blue. Certain parts of the nephridia can also be obtained in this way, especially the vesicle referred to later. The vesicle is always removed with portions of the nephridial tubes, but it is difficult to remove all the nephridium because the tubes are much coiled, and the nephrostome lies in the next segment in front as in all other Oligochaeta.

In preparing specimens for section cutting, various preserving fluids and staining reagents were employed. The killing process is a difficult one, as the contortions of the worm on the addition of any chemicals are very violent. The worm, if left to itself during the process, becomes so bent and shrunken that it is useless for sectioning. It is advisable, therefore, to hold the worm at both ends while it is being immersed in the killing fluid, and thus any serious contraction of the internal organs may be prevented. The great disadvantage of this method is that the brain and anterior part of the nerve cord are usually pressed out of shape, but it is easy to hold a few specimens nearer the middle, while killing them, when the anterior end can be obtained in fairly good condition.

For killing and preserving, Tellyeoniczky's aceto-bichromate was first used, but the tissues shrank badly in this reagent. I then tried Perenyi's fluid and Bles' fluid,* and these, while killing the worm very quickly, do not cause the tissues to shrink. Although, later on, other killing reagents were used, I found none so generally satisfactory as Bles' and Perenyi's fluids.

Zenker's fluid was used, especially for nephridia, with good results.

The staining reagents most commonly used were borax carmine with picro-indigo-carmine as a counter stain, and brazilin,† which is a very delicate and effective stain. Iron haematoxylin was also used, and this rendered the muscle fibres very distinct.

II. Historical Survey.

Before entering upon a description of the minute anatomy of *Tubifex rivulorum*, which forms the subject of this Memoir, it will be interesting to notice to whom we are indebted for the present condition of our knowledge of this form.

As early as 1745 Bonnet referred to it, but was content with describing certain peculiarities in the habits and general form of the worm, and with referring to its method of regeneration after artificial fission. He did not attempt to give any details of its structure. Schoeffer in 1764 gave a figure and description of *Tubifex rivulorum*, which he called "Kleinen Wasseraal."

---

* Bles' fluid ... ... 90 parts 70% alcohol.
7 ,, strong Formol.
3 ,, Glacial Acetic Acid.

† Brazilin ... ... 1% Iron Alum in 70% alcohol.
0·5% Brazilin.

O. F. Müller in 1773 classed it under Lumbricus as *L. tubifex*, but his description is very imperfect, and is largely corrected by D'Udekem. Lamarck (1816) separated *Lumbricus tubifex* and *L. lineatus* of Müller's Lumbricus and formed a new genus, Tubifex: the first he called *Tubifex rivulorum*, the second *Tubifex marinus*. Owing to apparent similarities in the structure of these worms and that of the Naiads, Lamarck united them in a class Hespides. In 1842 Hoffmeister recalled the attention of naturalists to the old "genus *Lombric*" of Müller, which he divided into three new genera, Lumbricus, Enchytræus and Sænurus, and he placed *Tubifex rivulorum* in his new genus Sænurus. It differed from the "genus *Lombric*" by the absence of a gizzard and by the fact that the setae are of unequal length. His observations on *Tubifex rivulorum* are very incomplete and inaccurate.

Grube in 1851 classed Tubifex among the Naiads under the name given to it by Hoffmeister—(*Sænurus variegatus*), and he assigned to it the following characters: "Ohne Kiemen, Borsten Bundelchen zweizelich, obere Borsten haar und hakenformig, selten obere und untere hakenformig, Blut lebhast roth oder roth gelb."

Budge (1850-51) has provided us with descriptions of the genital and respiratory organs. These descriptions are more accurate than those of Hoffmeister, but still very incomplete.

D'Udekem in 1855 published an account of the anatomy of the worm, dealing with all the systems in order, but studying them only in the living animal and in preparations made by teasing out parts of the body and then subjecting them to examination. His observations are certainly more detailed and accurate than those of earlier observers, but there are several inaccuracies, most of which have been noticed and corrected by still more recent observers. Many of the terms then in common use have since been discarded, and to some of these Beddard in his Monograph of the Oligochaeta refers, viz., the capsulogenous gland is now known as the spermatheca, the cloaca as the spermiducal gland, the vesicula seminalis as the sperm sac and the matrice as the egg sac.

In 1871 McIntosh published a paper in which he gives short accounts of certain of the organs, but no complete description of any system except the circulatory, as he concentrated attention on certain minor points such as the perivisceral fluid and corpuscles and the granular glands which form a complete investment around the intestine and dorsal vessel. His results will be considered in detail later.

Vejdovsky in 1884 published his well-known work "System und Morphologie der Oligochaeten." In this he makes frequent reference to Tubifex, and increases a good deal our knowledge of the structure of this worm. But in a work which embraces the whole of a large group it is impossible to describe in any great detail the structure of one member of that group. The same may be said of Beddard's Monograph of the Oligochaeta, published in 1895.

Several other papers have been inserted in the Bibliography at the end of this work. Some of these deal only with one small part of the subject, and reference will be made to them in the right places. Others have been mentioned in the list because they have some more distant bearing on the work, but yet are sufficiently allied to it to be of interest.

It has seemed advisable to revise all this scattered work, and, by a careful and detailed investigation of the worm, to build up a Memoir which shall give, as far as possible, a full account of the structure of this form, together with any other points of interest which may arise during the investigation.

This paper, previous to its publication, was presented as a thesis for the D.Sc. degree of the University of London, and in this connection I wish now to express my gratitude to Professor Jackson who kindly photographed the drawings for me. It is with pleasure also that I acknowledge my indebtedness to Professor Dendy for valuable suggestions made while the work was in progress and for careful criticism of the finished work, which was carried out under his supervision in the Zoological Laboratory at King's College.

III. General Remarks.

Tubifex rivulorum is found in large numbers in the mud of the estuarine Thames, where, at low water, they may often be seen as bright red masses. In every consignment of mud which was examined this species was always found associated with another worm—*Limnodrilus udekemianus*—both belonging to the family Tubificidae. The two worms can easily be distinguished from one another if they are examined under a low power of the microscope; but, with practice, it is also possible to separate them when in the mass. One notices that the anterior segments of all of them are reddish in colour, but there is a marked difference in the posterior segments of the two forms. In the case of *Tubifex rivulorum* the posterior segments also are red in colour, but in *Limnodrilus udekemianus* the red colour is masked by the presence of pigment in the body wall. The pigment is yellow or orange in colour, and is confined to a narrow band in each segment, forming an incomplete ring around it and giving the worm a striped appearance.

If specimens of both worms are examined under the microscope, it will be seen that the setae are different, Tubifex showing two kinds of setae, capilliform and sigmoid, while Limnodrilus has only sigmoid.

If the receptacle in which the worms are being kept is suddenly jarred with a sharp knock, they all simultaneously contract, hiding themselves almost completely in the mud. *Tubifex rivulorum* is also sensitive to light, for, if a bright beam from an arc lamp be projected through the water on to the mass, all the worms belonging to this species contract instantly and disappear. *Limnodrilus udekemianus* appears to be unaffected by the light, for the posterior end of the body still continues to wave about in the water.

If individuals of both species be placed on a slide, it is possible to distinguish them by their movements. *Tubifex rivulorum* is much more rapid in its movements, and retains its activity much longer than *Limnodrilus udekemianus* after being deprived of the mud in which it lives.

EXTERNAL CHARACTERS.

Tubifex rivulorum is a small worm varying from 30-50 mm. in length, and is of a bright red colour. The colour is due to the presence of red blood, which is clearly visible through the transparent body wall. The worm is bluntly pointed at both ends, the widest part of the body being in the anterior third. The body is divided throughout its length into a number of segments, all quite clearly marked off from one another, and decreasing in size towards the posterior end. The number of segments in a fully-developed worm varies from 112 to 130. The worm never exhibits any "secondary" annulation of the segments, such as occurs so generally in Limnodrilus.

The Prostomium:

This appears as an outgrowth or process of the first segment. It is conical in shape with a slightly blunted apex and overhangs the mouth on the dorsal surface. It is separated from the first segment by a transverse furrow. It is extremely sensitive, and is undoubtedly used as an organ of touch, and consequently is plentifully supplied with nerves which arise from the brain.

Setae:

The setae, as in all Oligochaeta, are the organs of locomotion. They consist of chitinous rods derived from specialised cells of the epidermis. Part of each seta is buried in the body wall, and may project into the body cavity, but the rest protrudes beyond the surface of the body.

The development of these organs has been carefully observed and described by earlier writers, especially Vejdovsky, who first established the fact that all setae are ectodermal in origin. A varying number of setae are implanted in "setigerous sacs or follicles" which arise as invaginations of the epidermis. The arrangement of the setae in a sac is like that of the sticks of an open fan (Pl. II, fig. 5). Each seta has its origin at the base of the sac, but it is lodged in a separate cavity and divided from its fellows by a small piece of the body wall. The cuticle, which forms the outermost layer of the body wall, is continued into each of the cavities, and forms a lining to it for the greater part of its length. Near the blind end of the sac the cuticle is absent, and is replaced by a large group of ectodermal cells whose boundaries are indistinct, but whose nuclei are large, round and nucleolated. According to Vejdovsky, any of these cells are capable of giving rise to new setae to replace those which may have been lost. The free ends of the setae are seen constantly to change their position as the worm rapidly coils and uncoils itself. Sometimes they are directed forwards, then backwards, and often, especially when the worm is quiet for a moment, they are placed almost at right angles to the body. When the worm is crawling forwards the setae are always pointing somewhat backwards, but a sudden twist of the body is sufficient to change their position completely.

There are two sets of muscles by which the setae are moved :—

(*a*) The parieto-vaginal muscles, which are attached to the base of the setigerous follicle and to the body wall on all sides of it. These muscles regulate the movements of the whole follicle. If those lying on the posterior side of the sac contract, the inner ends of the setae are drawn back, and the free ends are pushed forward, whereas, if those muscles on the anterior side contract, the setae are directed backwards (Pl. II, fig. 5, *m.p.*).

(*b*) The intrafollicular muscles (fig. 5, *m.f.*). These are attached to the body wall and to the individual setae, and thus the movements of the setae can be regulated.

The development of the setae in this worm resembles, in all essentials, that of the setae of other Oligochaeta, and therefore it is only necessary to refer to it briefly. The setae appear as small cones of chitinous substance at the bottom of the sac. The apex of the seta is first developed and then growth in length proceeds from the inner end until the seta has pierced the body wall and attained its full length.

The setae are arranged in four bundles in every segment of the body except the first and the last. Two of these bundles are ventral in position, and lie one on either side of the mid-ventral line. These are spoken of as the ventral bundles (Pl. III, fig. 11, *v.bl.*). The other two are much more dorsal in position, and are referred to as the dorsal bundles (fig. 11, *d.bl.*).

Three kinds of setae are present, viz., capilliform, uncinate, and pectinate (Pl. II, fig. 6). The distribution of the capilliform setae is limited, for they are confined to the dorsal bundles, and do not usually extend further back than the 30th segment. The uncinate setae are common to both dorsal and ventral bundles throughout the body, while the pectinate form is limited to the dorsal bundles of the anterior segments, and is absent from some of these.

The capilliform setae are long, straight, hair-like in form, and narrowing to an extremely fine point at the free end (Pl. II, fig. 6, *d*). By far the greater part of the seta is exposed, only about a quarter of it being embedded in the setigerous sac. These setae are quite smooth and devoid of barbs, such as are described by Beddard (1895) as occurring in *Lophochaeta ignota*. The longest ones occur in segments 6 to 9; behind the latter segment they become smaller and smaller, until, at last, they completely disappear. It is very unusual for there to be more than three capilliform setae in a bundle, and very commonly only one or two are present. In those cases where three setae are present, they vary considerably in length, and as a rule two of them are long while one is much shorter.

Beddard (1895), in his classification of the setae of Oligochaeta, divides them into two main groups:—

(*a*) Long, slender setae gradually diminishing in diameter towards the pointed extremity—capilliform.

(*b*) Shorter setae of a curved form, something like an elongated S with a thickening at about the middle—sigmoid setae.

The uncinate and pectinate setae of *Tubifex rivulorum* belong to this latter class. Of these, the uncinate form is much more common than the pectinate. They are both of the same general shape, but the thickening referred to by Beddard is not very strongly developed. Neither does it occur at the middle of the seta, but is always nearer the outer free end and approximately divides the seta into a distal third and a proximal two-thirds (Pl. II, fig. 6, *a*) In the uncinate setae the distal end is bifid, but the two prongs vary somewhat in size and shape in different setae. The uncinate setae in the dorsal bundles have the two prongs equal in size and of the same shape, that is, somewhat narrow throughout and tapering gradually to a very fine point at the distal end (Pl. II, fig. 6, *b*). In the ventral bundles, where the uncinate setae only are present, the two prongs are of slightly different size and shape. The dorsal one is somewhat slender, and comes to a sharp point at the tip. The ventral one is shorter than the dorsal, and of a blunter nature, its apex being somewhat more rounded (Pl. II, fig. 6, *a*). There is never, however, such a marked difference in 'he size of the prongs as in the case of Limnodrilus, where the ventral one is always much shorter than the dorsal.

The number of uncinate setae which may be found in a bundle differs somewhat in different regions of the body. For instance, in both dorsal and ventral bundles of the first two setae-bearing segments there are never more than two setae, and these are very small compared with those which come close behind. In segments 3 to 9 or 10 they reach their maximum size, and there are often four or five present in the ventral bundles, but rarely more than three in the dorsal ones, where they are accompanied by capilliform and sometimes by pectinate setae. More posteriorly they decrease in size and number, soon dwindling down to two in a bundle, and near the posterior end of the body it is usual to find only one small seta in each bundle.

The pectinate setae are, as already mentioned, less numerous than the uncinate type, and are confined to the dorsal bundles of the anterior segments. There, therefore, it is possible to find in the same bundle setae of three different kinds: capilliform, uncinate, and pectinate, the total number rarely exceeding six. The pectinate setae resemble the uncinate of the dorsal bundles in general form. The only difference between them is the presence, in the pectinate form, of subsidiary prongs between the two main divisions of the seta (Pl. II, fig. 6, *c*). These subsidiary prongs are figured and described by McIntosh, Lankester and Beddard. Their number varies from 1 to 4, the latter number occurring most rarely.

I have not yet been able to identify the webbed setae described by Lankester (1871). He discusses at some length the form of the sigmoid setae in this worm. He states that he has often seen sigmoid setae, in the dorsal bundles of the first ten segments, which are provided with a web between the two prongs. Benham, who also investigated this species, could not identify the web. During my observations on the setae I have particularly looked for the appearance of this web, but have always failed to find it. A possible explanation of this difference of opinion is that Lankester was looking at the pectinate setae which do occur in the dorsal bundles of these anterior segments only, and mistook the subsidiary prongs for a continuous web stretching across between the two main prongs. Again, Lankester recounts how he has seen a number of hairs (6 or 7) surrounding certain of the setae near the apex. He states that they result from the splitting up of the horny substance of the seta. He goes on to describe how a number of small dark particles are placed at intervals along the hairs. It is quite easy to recognise the condition that he describes, but it seems to me that his observations may bear another interpretation. When the worms are kept in the laboratory for a time, even though they are placed under running water, they become infested with fungus growths. These have the form usually of long, delicate filaments which appear to have their origin between the prongs of the setae, and present exactly the appearance described by Lankester.

THE BODY WALL.

As in most Oligochaeta the body wall consists of the following layers:—(1) Cuticle, (2) Epidermis, (3) Circular Muscles, (4) Longitudinal Muscles, and (5) Peritoneal Epithelium.

1. *The Cuticle* is a delicate layer of non-cellular nature which lies outside the epidermis, and is formed as a secretion from the epidermal cells. It completely invests the body, and is about 3 μ thick.

2. *The Epidermis* consists of a single layer of cells throughout, but varying somewhat in thickness in different parts of the body. For purposes of description we may divide the epidermis into:—(*a*) Ordinary or extra-clitellar epidermis; and (*b*) Clitellar epidermis.

(*a*) *Ordinary or Extra-Clitellar Epidermis.* The ordinary epidermis consists of two well-marked types of cells, viz., gland cells and columnar supporting cells, and is from 6 μ to 8 μ thick. The gland cells are large, somewhat irregular in outline, and with the nucleus, as a rule, situated in the lower half of the cell. The cytoplasm of these cells is densely granular. The nuclei are rounded or slightly oval in shape, and each contains a nucleolus (Pl. II, fig. 5, *hyp.*).

The gland cells are separated from one another by the columnar epidermal cells, or "supporting cells" as Atheston (1898) calls them. They are narrower than the gland cells, generally columnar in outline with the broader end lying next to the cuticle. The nuclei are oval in shape, quite conspicuous, and situated near the middle of the cell.

(*b*) *Clitellar Epidermis.* The clitellum does not develop until the worm has nearly reached sexual maturity. It then becomes differentiated from the ordinary epidermal cells, and is confined to the principal reproductive segments, viz., segments 11 and 12. It has the form of a complete girdle, but is less well developed on the ventral than dorsal surface. It merges gradually into the ordinary epidermis anteriorly and posteriorly. It is discontinuous, of course, at the openings of the setigerous follicles, the spermathecal pore and penis.

It is composed of a single layer of cells throughout. At first, the cells are little different from the ordinary gland cells of the epidermis. The nucleus is a conspicuous rounded body, situated at the base of the cell, and exhibits a very well-marked reticulum with a nucleolus. The cytoplasmic contents of the cell are finely granular.

This is the usual structure of the clitellum, even in individuals which appear to be quite mature, and, consequently, this is the condition which has been described by most observers. Atheston (1898), for example, says of the gland cells of the clitellum that they are smaller and more numerous than those of the ordinary epidermis, otherwise they are similar.

Ekitaro Nomura (1913), in his description of *Limnodrilus gotoi*, gives a short account of the clitellar gland cells as they occur in that worm. He says that the gland cells are 20-23 μ long, that is, four times the length of the ordinary clitellar cells, and 8-10 μ across. "Three well-marked stages can generally be observed in mature specimens: a highly vacuolated condition, a more or less granulated condition, and one in which the cells contain many globules."

In most of the specimens of *T. rivulorum* that I examined the clitellar gland cells were only a little larger than the ordinary gland cells, and the contents were granular. In a few cases, however, the clitellum was enormously enlarged, and I can only suppose that the maximum development of the clitellum is not reached until a very short time previous to the formation of the cocoon. The cells at this time may be as much as 40 μ to 45 μ long, and 11 μ to 14 μ across (Pl. III, fig. 12). They are almost oblong in shape, tapering just a little at the inner end. The nuclei are still visible, but are much less distinct. They remain near the inner end of the cell. The increase in size of the cells at this time is accompanied by the deposition in them of a large number of globules of the secretion which forms the cocoon. Many of the cells are quite full of the secretion, which masks, almost completely, the cytoplasmic contents. In others, where the secretion is present in smaller quantities, it is arranged fairly regularly in the form of rounded masses, 2 μ in diameter, which congregate principally near the lateral walls of the cell, where they are arranged in longitudinal rows (Pl. III, fig. 12, *se. c.*). The cytoplasm is somewhat vacuolated. Between these enlarged and modified gland cells are supporting cells, which are as long as, but much narrower than, the gland cells. The nuclei of these cells are oval in shape, and may be situated in any part of the cell (fig. 12, *in. c.*). Certain fibres from the longitudinal muscular layer may turn outwards and come into close contact with the inner ends of the gland cells.

3. *The Circular Muscle Layer* lies just within the epidermis. It is an extremely delicate layer, and is with difficulty recognised in transverse and longitudinal sections. Occasionally it can be fairly clearly seen in an oblique section, when the individual fibres appear as incomplete bands surrounding the body (fig. 5, *c. m.*).

4. *The Longitudinal Muscles* are better developed than the circular, and can be seen in longitudinal sections of the body wall as elongate fibres. Seen in transverse section they have the appearance of flat plates or lamellae embedded in a granular substance. There is no axial core, as is so characteristic of the longitudinal muscles of Lumbricus, neither are they divided into dorsal and ventral regions by lateral lines, as is described by Nomura (1913) for *Limnodrilus gotoi* (fig. 5, *l. m.*).

5. *The Peritoneal Epithelium* consists of a single layer of somewhat flattened cells with prominent rounded nuclei.

COELOM AND COELOMIC FLUID.

The coelom of *Tubifex rivulorum* is, as is usual in the Oligochaeta, well marked and spacious, and divided into a series of compartments by septa. The number and arrangement of the septa correspond to the external segmentation of the body. The septa between segments 1 to 4 are incomplete. The coelom only communicates indirectly with the exterior—by means of the nephridia and genital ducts. There are no dorsal pores such as are found in earthworms. The coelom shows no division into different cavities, other than that due to segmentation, with the exception of the egg sac and sperm sac, which are simply portions of the coelom bounded by special walls and arising as simple outgrowths of the coelom of certain segments. These organs will be described in detail with the rest of the reproductive system.

The coelom is lined throughout by the delicate peritoneal epithelium which is also reflected over the other organs in the body cavity. The shape and size of the cells forming this epithelium vary considerably in different parts of the body, but for the present these modifications in its structure will only be mentioned briefly. The parietal and visceral layers are those which form the innermost layer of the body wall and the outermost layer of the intestinal wall respectively. The cells of the parietal layer have already been described. The visceral layer is formed chiefly of much enlarged pear-shaped cells—the "chloragogen cells" of Claparède. This applies to the visceral layer around the intestine. In the region of the buccal cavity, pharynx and oesophagus, the cells of this layer very nearly resemble those of the parietal layer. In certain regions of the nephridial tubes the peritoneum is composed of very large, vesicular cells which are very easily detached from the tube and from each other. In other parts of the tube, chiefly those nearer the nephrostome and nephridiopore, the ordinary, flattened epithelial cells are present.

The coelom is always filled with a colourless coelomic or perivisceral fluid, by which all the organs of the body are constantly bathed. Suspended in the fluid are a number of colourless corpuscles which may be described as coelomic or perivisceral corpuscles. The number of these corpuscles in different individuals varies enormously, but two kinds are usually recognisable—amoeboid and spherical. In some individuals the spherical corpuscles are much more numerous than the amoeboid, while in others they are more evenly distributed. It is often rather difficult to decide whether a particular corpuscle is in the amoeboid or spherical condition. It is not really actively amoeboid, yet it is irregular in outline and with no clearly defined contour. It would seem, therefore, that transitional stages may occur at such times when an amoeboid corpuscle, having, as Beddard suggests, become loaded with granules of an excretory nature, ceases to be amoeboid and gradually becomes spherical in outline. Both kinds of corpuscles are granular, the granules sometimes being of a yellowish hue.

THE ALIMENTARY CANAL.

The general arrangement of the various regions of the alimentary canal can be clearly seen if the living worm be examined under the microscope, owing to the transparency of the body wall. If examined in this way, it will be seen that the wall of the alimentary canal in the first five segments is almost colourless, or only slightly yellowish in colour. In segment 6, however, the appearance changes very suddenly, for the wall has now a blackish or brownish hue, due to the presence of specialised cells of a glandular nature known as chloragogen cells, which will be described in detail later. More posteriorly the colour again changes, becoming gradually lighter and lighter until in the region of the anus the wall of the alimentary canal has again a pale yellow colour. As in all Oligochaeta, the alimentary canal has the form of a straight tube extending from the mouth, situated on the ventral surface of the first segment, to the anus, which is surrounded by the last segment of the body, or the anal segment.

The alimentary canal can be divided into the following regions:—(1) mouth, (2) buccal cavity, (3) pharynx, (4) oesophagus, (5) intestine, and (6) anus.

1. *The Mouth.* The mouth is ventral in position, surrounded by the first segment of the body or peristomium, and overhung by the prostomium. When closed it appears as a transverse slit bounded by a slightly puckered wall, but when it is open the aperture is rounded (Pl. II, fig. 4, *mo.*).

2. *Buccal Cavity.* The mouth leads into the buccal cavity, which is short—only extending through the first segment of the body. It is partly covered by the cerebral ganglia, dorsally. The buccal cavity is capable of extrusion, but this does not seem to take place very often under ordinary conditions. I have, however, frequently observed it when ether has been added to the water in order to quiet the worm when it is under observation. The buccal cavity can then be seen as a somewhat frilled organ protruding from the mouth. When once the cavity has been extruded, it is not readily drawn in again.

A transverse section of the buccal cavity, when not extruded, shows it to be of wide calibre, with a straight, unfolded wall. The latter is very thin, and is composed of a single layer of epithelial cells, somewhat cubical in shape, with well-marked nuclei and numerous short cilia. Outside this layer of epithelial cells a few muscle fibres are scattered, but they are not sufficiently numerous to form definite muscular layers (Pl. II, figs. 4 and 7, *bu.c.*).

3. *The Pharynx.* Leading out of the buccal cavity is the pharynx, which extends through segments 2 and 3.

It is surrounded by the peripharyngeal connectives which connect the cerebral ganglia, lying dorsally, with the sub-oesophageal ganglion or anterior end of the ventral nerve cord. The pharynx can never be everted, and is provided with exceedingly muscular walls, the musculature being chiefly dorsal in position. The lumen is folded and ciliated, the cilia being considerably longer than those of the buccal cavity (Pl. II, fig. 4, *ph.*).

4. *The Oesophagus.* The pharynx opens into the oesophagus, which also extends through two segments (4 and 5), and communicates at its posterior end with the intestine. The oesophagus is a narrow tube, its lumen somewhat folded and ciliated. The wall is thin, consisting of a ciliated epithelium internally and a few muscle fibres externally, arranged in circular and longitudinal directions, the circular muscles inside (fig. 4, *oe.*).

Situated in those segments occupied by the oesophagus, and, to a less extent, those occupied by the pharynx, are organs of a glandular nature known as septal glands. These organs occur in many Oligochaeta, but are specially well developed in aquatic forms, although they have been described by Vejdovsky as being very well marked in *Allolobophora foetida.* These glands are attached primarily to the septa, but may extend on to the walls of the oesophagus, and to a large extent lie freely in the body cavity. Each gland is a mass of pear-shaped cells, the narrower part of each being prolonged into a duct which opens into the oesophageal region of the alimentary canal. The cells possess very distinct nuclei, situated in the broader part of the cell (Pl. II, fig. 4, *s.gl.*).

5. *The Intestine.* The intestine commences in segment 6, and extends throughout the entire remaining length of the body to the anus, which, as already stated,

opens to the exterior on the last segment (Pl. II, fig. 4, *in.*). It is overlaid in the anterior segments by the supra-intestinal blood vessel, and more posteriorly by the dorsal vessel, and itself covers the ventral vessel which lies freely in the body cavity. The intestine is kept in place by the septa, which constrict it slightly at intervals, and by special muscles which pass from it to the body wall. Its lumen is larger than that of the oesophagus, and is unfolded. It is ciliated throughout, the cilia being specially long and abundant in the posterior segments of the body, especially the last six or seven segments.

The structure of the intestinal wall from within outwards is as follows:—

(*a*) A single layer of somewhat elongated cells forming the ciliated epithelium. The cilia are not very conspicuous except at the anterior and posterior parts of the intestine (Pl. III, fig. 13, *ct.ep.*).

(*b*) A very thin muscular layer composed of a number of isolated circular (on the inside) and longitudinal (on the outside) muscle fibres (fig. 13, *c.m.*; *l.m.*).

The wall of the intestine is very vascular, and the blood vessels are situated between the ciliated epithelium and the layer of circular muscles (fig. 13, *b.v.i.*). These vessels form what is known as the intestinal network (see circulatory system).

(*c*) A glandular layer composed of a very large number of unicellular glands which cover the entire surface of the anterior half of the intestine, and also cover the dorsal blood vessel (fig. 13, *c.c.*). It is to the presence of these glands that the dark colour of the intestinal wall is due. They were called chloragogen cells by Claparède, and this name is still applied to them. When examined in section, they are seen to be somewhat club-shaped cells with a fine external membrane enclosing a large number of granules of two kinds, some being much larger than the others, and of a dark brownish hue. Both kinds of granules are very inert, and stains and other reagents have little or no effect upon them.

If the body wall of the worm be ruptured, these cells, which are very easily dislodged from the wall of the intestine, can be liberated into the water. In the water they swell considerably, and the two kinds of granules which they contain can be clearly seen (Pl. III, fig. 14). The larger ones tend to congregate near the centre of the cell, the outline of which becomes rather indistinct and shadowy when the cell is immersed in water. The smaller granules can be more clearly seen near the periphery of the cell where the larger granules are not so abundant. All the granules of a cell which has been freshly liberated from the body of a living worm exhibit active molecular movements, and this movement is kept up for some time if the wall of the cell is not broken. Gradually, however, this molecular movement becomes slower and slower until it finally ceases. The granules then appear to congregate at one part of the cell, sometimes at the side, sometimes nearer the middle, the rest of the cell being quite clear and transparent. Probably this massing together of the granules heralds the breaking down of the cell.

All these points in the structure of the chloragogen cells can be made out in the fresh material. This amount of investigation was carried out with considerable care by McIntosh, and his results were published in his paper entitled "On some points in the structure of Tubifex" (1871). In quoting Dr. Buchholz' paper "Beiträge zur Anat: der Gattung Enchytræus" (1862), McIntosh mentions that this author has put a distinct nucleus in all his figures of the chloragogen cells of Enchytræus. McIntosh appears to have made efforts to demonstrate the presence of a nucleus in the fresh, unstained chloragogen cells of Tubifex, but owing, as he says, to the large number of granules always present in the cell, he was not able to do this. It certainly does need considerable care in observation in order to see the nucleus, but it is comparatively easy to do so if the cells are floated on to a large quantity of water on a slide and a cover-glass put on. The cells then roll about in the water, and during their movements it is often possible to see the nucleus. McIntosh also states that even after careful examination of transverse and longitudinal sections of the worm he arrived at the same result. I find, however, that in any section of the intestinal part of the worm, which has been appropriately stained, viz., with borax-carmine, the nuclei can be very plainly seen, as they are large and each has a distinct nucleolus (Pl. III, fig. 13).

The true shape of the cells is better seen in sections than by examining them in the fresh condition. In sections they are seen to be truly pear-shaped, the broader end lying freely in the body cavity. In such preparations the nucleus, which is oval in shape, is usually situated in the narrower part of the cell. It is quite useful to make permanent smears of these cells and stain them on the slide. A stain which differentiates the nucleus particularly well is Brazilin. In these smears the granules stain very slightly: not nearly so heavily as does the nucleus (Pl. III, fig. 15).

Rice (1902) has published a paper on the chloragogen cells of *Lumbricus herculeus*, and he enters in some detail into their origin, growth and structure, and gives some suggestions as to their function. It is evident from his descriptions that their growth and structure are very similar to those of the chloragogen cells of Tubifex. Rice carried out a series of feeding experiments, and found that neither excess of food, nor starvation, nor a varied diet, had any effect upon these cells. He also pointed out that the granular contents are apparently lifeless, for no stain appreciably affects them, nor are they altered in any way by the addition of strong acids. The lifeless condition of the adult cell suggested to Rice the possibility that these cells have performed their function in the young worm—presumably they would have some connection with the elaboration of food, as they cover the dorsal vessel as well as the intestine—and have become functionless in the adult. This theory is supported by the fact that these cells in a very young worm exactly resemble those of the ordinary peritoneum. In the larger worms a gradual development into typical chloragogen cells can be traced. The cells increase in length, the characteristic granules appear, and they become less and less responsive to stains, with the exception of the nucleus which stains throughout. Earlier observers believed these cells to be secretory in function, but, judging from their lifeless condition in the adult, their function, if any, would surely be one of excretion rather than secretion.

CIRCULATORY SYSTEM.

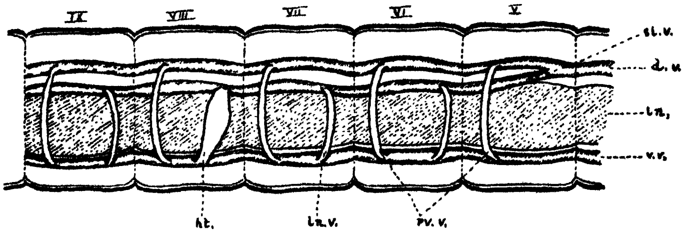
The circulatory system, as in all other Oligochaeta, is a closed one, having no communication with the coelom. It consists of a series of main vessels having a longitudinal direction, and united with one another by lateral vessels in each segment. The blood vessels are well-developed tubular structures, and their walls are very delicate. In none of the vessels does there seem to be an epithelial lining. Its place in these lower forms is taken by a delicate membrane, destitute, apparently, of any cellular structure. Beddard described the same condition in other aquatic worms, viz., Naiads and Enchytræidae. It is only in the true earthworms that this membrane is replaced by an epithelium consisting of large and conspicuous cells. In the smaller vessels, such as the periviscerals and intestinals, the wall consists of nothing more than this membrane covered externally by a single layer of flattened, peritoneal cells. Such branches must necessarily be non-contractile. The walls of the main blood-vessels show a rather more complicated structure, but still remain very thin and delicate. The membrane, mentioned above, is supported externally by a few muscle fibres. The circular muscles are very few, and are situated just outside the membrane. The longitudinal fibres are better developed, and lie just underneath the peritoneal epithelium, which forms the outermost covering of the wall.

Owing to the rapid movements of the worm, the dorsal and ventral vessels are constantly thrown into a number of zig-zags or S-shaped portions, which are rendered much more apparent by the addition of chloroform or ether to the water in which the worm is placed, when the movements of the worm become very violent.

The main trunks which have a longitudinal direction are the dorsal, supra-intestinal, and ventral vessels.

1. **The Dorsal Vessel** extends through the entire length of the body from the anal segment to the prostomium (Pl. I, fig. 1, *d.v.*). It lies dorsal to the alimentary canal for the greater part of its length, but in the region of the reproductive organs it changes its position and comes to lie nearer the ventral vessel. Further back it reassumes its original position.

2. **The Supra-Intestinal Vessel** is always attached to the dorsal wall of the intestine, and is invested by a layer of chloragogen cells continuous with those which form the outermost layer of the intestinal wall (Pl. III, fig. 9, *si.v.*). It originates in segment 5 as an offshoot of the dorsal vessel which lies above it, and it extends through the body to a short distance behind the segments containing the reproductive organs. Nomura (1913) states that in *Limnodrilus gotoi* this vessel opens into the dorsal vessel again at the posterior part of the body. Although I have carefully examined many serial sections, I have not been able to find this second opening of the supra-intestinal into the dorsal vessel in *Tubifex rivulorum.* The vessel seems rather to diminish gradually in size, and finally to disappear. In those segments of the mature worm which contain the reproductive organs, the supra-intestinal vessel is slightly displaced and lies a little to one side of the mid-dorsal line. This is probably due to the pressure exerted by the reproductive organs on the other organs in the body.



TEXT-FIG. 1. Diagram of segments *V—IX* showing the positions of the principal blood vessels with their connections in these segments. *d.v.* dorsal vessel, *si.v* supra-intestinal vessel, *in.* intestine, *v.v.* ventral vessel, *pv.v.* periviseral vessel, *in.v.* intestinal vessel, *ht.* heart.

3. **The Ventral Vessel** extends through the whole length of the body, and lies beneath the alimentary canal between it and the nerve cord (Pl. I, fig. 1, *v.v.*). It

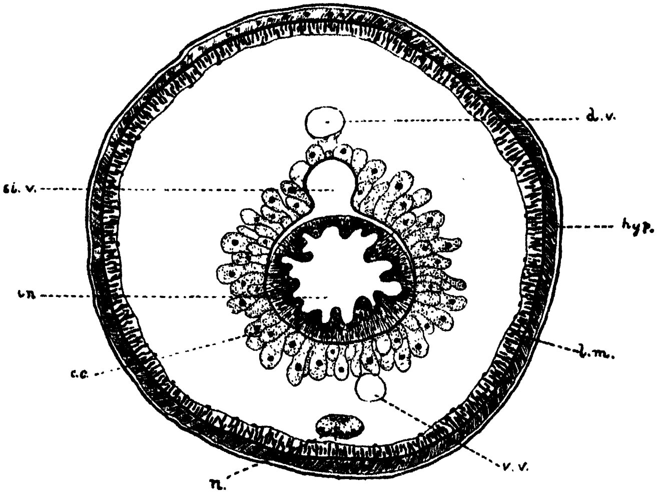
commences in segment 1, where it is paired, its two parts uniting with the two branches into which the dorsal vessel divides, also in segment 1. These two parts of the ventral vessel pass backwards as two converging trunks as far as segment 3, where they unite. It is attached to the intestine throughout the greater part of its length, but occasionally appears to lie freely in the body cavity. It should be noticed that the nephridia of the posterior segments of the body are situated close to the ventral vessel with the walls of the nephridial tubes closely pressed against it. In the last segment of the body the dorsal and ventral vessels unite and are slightly coiled.

These longitudinal trunks are connected with one another by a series of commissural vessels. These are of two kinds: (*a*) intestinal, (*b*) perivisceral or coelomic.

(*a*) *Intestinal Vessels.* The principal intestinal vessels, of which there is a pair to each segment, connect the supra-intestinal with the ventral vessel and are well developed in all segments behind the fifth. They have their origin from the supra-intestinal vessel near the middle of the segment and well in front of the perivisceral trunks (Pl. VI, fig. 42, *in. v.*). These intestinal vessels do not lie freely in the body cavity but pass beneath the layer of chloragogen cells, and thus encircle the intestinal wall. In the posterior segments they are connected with the dorsal instead of the supra-intestinal vessel.

In addition to this principal intestinal trunk, there are accessory vessels, which are, however, much less conspicuous (fig. 42, *in.v.*^{1}). These are best seen by treating the living worm with ether on a slide. At first, this has the effect of causing strong contractions of the intestine and blood vessels, which rather hinder than aid the observer. But if the worm is allowed to remain on the slide for some time these contractions become less violent, as the animal becomes exhausted, until, finally, all movement ceases except the occasional contraction of the intestine. The body of the worm becomes somewhat flattened, and the chloragogen cells, which at first completely hide the underlying tissues, become clearer. It is then possible to investigate the blood vessels which pass to the intestine wall. These branches become filled with blood, and, if the worm is lying on its side, are comparatively easy to see. The number and arrangement of these smaller intestinal vessels vary in different parts of the body. The largest vessel, which has already been described, is constant. The variable ones are the accessory intestinal branches which lie between the intestinal proper and the perivisceral trunk (Pl. VI, fig. 42). In the anterior segments of the intestinal region of the body there may be as many as five pairs of accessory intestinal vessels, all branched and forming a vascular network over the intestinal wall, but they do not appear to have any connection with the ventral vessel. In the posterior segments these vessels gradually decrease in size and number, and become very difficult to follow.

(*b*) *The Perivisceral Vessels* are present in all segments of the body except segment 8. They arise in pairs from the dorsal vessel near the posterior border of each segment, and are connected with the ventral vessel below. These are quite large trunks, passing out almost at right angles to the dorsal vessel, and form in most segments a series of complex coils, often extending from one end of the segment to the other (Pl. VI, fig. 42, *pv. v.*). This coiling of the perivisceral vessels allows of ample freedom of motion for the worm—a very necessary precaution in view of its rapid and sudden movements. They do not branch, and, therefore, there is no integumental network in this worm, such as is described by Beddard as occurring in Ilyodrilus and Bothrioneuron. In the anterior segments of the body the perivisceral trunks lie freely in the body cavity. More posteriorly, particularly in that part of the body which is waving about in the water, these vessels, while not branching, are always pressed closely against the body wall for a good part of their length, and remain in this position permanently. This would suggest that aeration of the blood takes place through the body wall, which is very thin near the posterior end of the body. At its anterior end the dorsal vessel divides into two branches, which become slightly coiled and then dip down ventrally to become continuous with the anterior ends of the ventral vessels. In segment 2 the perivisceral vessels are given off from the dorsal vessel near the posterior border of the segment.



Text-Fig. 2. Diagrammatic tranverse section through segment *IX* to show the relative positions of the principal blood vessels. *hyp.* epidermis, *l.m.* longitudinal muscles, *c.c.* chloragogen cells, *in.* intestine, *si.v.* supra-intestinal vessel, *d.v.* dorsal vessel, *v.v.* ventral vessel, *n.* nerve cord.

Instead of passing out at right angles to the dorsal vessel, as is the rule, they have a somewhat more forward direction and may extend into the segment in front, the septum between segments 1 and 2 being absent (Pl. VI, fig. 41, *pv. v.* 2). They become slightly coiled, and then dip down to open into the ventral vessel a short distance behind their origin from the dorsal vessel.

The perivisceral vessels of segments 3-7 have a similar distribution to that described above for segment 2, but in segments 4-7 they lie entirely in the segment to which they properly belong, owing to the presence of septa between adjacent segments, thus preventing the passage of the vessels from one segment to the other. It should be noted also that the right and left periviscerals of segments 2 and 3 open into the right and left branches of the ventral vessel respectively.

In segment 8 the perivisceral vessels are absent. In segments 9 and 10 they become coiled around the anterior and posterior sperm sacs respectively. In segments 11-17 of a mature worm which contain the ovisac the periviscerals are always coiled around this organ. In all the segments behind those containing the reproductive organs these vessels pass simply from the dorsal to the ventral vessel.

The "hearts" are situated in segment 8. They originate from the supra-intestinal vessel above, in the posterior part of the segment, and open into the ventral vessel below (Pl. I, fig. 2, *ht.*). They are much enlarged, especially near their origin from the supra-intestinal trunk, and are contractile.

The blood is a red, non-corpusculated fluid, the haemoglobin being dissolved in the blood plasma.

THE NERVOUS SYSTEM.

The nervous system of *Tubifex rivulorum* is formed upon the same plan as that of all Oligochaeta. It consists of cerebral ganglia united to a ventral chain of ganglia or nerve cord by peripharyngeal connectives. As in most other Oligochaeta the whole nervous system lies completely in the body cavity. It is possible to examine the arrangement of the ganglionated chain and peripheral nerves in the living worm, especially after the addition of ether to the water in which the worm is lying. The ether increases the transparency of the body wall so that the internal organs can be seen more easily, and in some cases the alimentary canal becomes so contorted that the ventral nerve cord is left quite freely exposed for a considerable distance. This is a very useful method of investigation, as it enables one to compare the form and size of the ganglia, and the proportion of connective to ganglion in the segment in different parts of the body, and these proportions vary a good deal. It is, however, of very little use for a detailed examination of the brain. In the living worm it is usually very difficult to define the outlines of the brain, even after the addition of ether. It has happened, however, very occasionally, that I have been able to see the brain quite clearly by this method. The difficulty which is usually experienced in examining the brain in the living condition is due partly to the greater transparency of the brain compared with that of the body wall, and partly to the contractions of the pharynx over which the brain is situated. The great drawback to the use of ether is that the blood vessels in the anterior region of the body become somewhat distended and quite full of blood. This, of course, only serves to obscure the brain more.

Even though one may be fortunate enough occasionally to get a good idea of the shape of the brain, it is better to complete the investigation by means of serial sections, which are, of course, necessary for a proper examination of histological details, both in the brain and ventral nerve cord. Many attempts were made to make use of *intra-vitam* methods of staining, as it was hoped that the nervous system would be rendered more distinct when the tissues were stained in the living condition. These attempts, however, were not successful, as the stain (methylene blue) penetrated the body wall very slowly and incompletely, and did not reach the nervous system at all.

The Cerebral Ganglia, as in most aquatic Oligochaeta, lie far forwards, just behind the prostomium. Their posterior border lies at the level of the boundary between the buccal cavity and pharynx. They are dorsal in position, and form a comparatively large brain, the structure of which is complicated by the presence of several lobes. The brain is concave behind and in front, and consists of a solid mass of nerve fibres and nerve cells. Anteriorly it is produced into three horns or lobes, two lateral and one median: the former may be described as the antero-lateral lobes, and the latter as the anterior median lobe (Pl. I, fig. 1, *an.l.*; *m.l.*). From these, nerves arise which will be considered later. The anterior median lobe is characteristic of the family Tubificidæ, but is not always present as a simple lobe. In a few more highly specialised forms, *e.g.*, Bothrioneuron, it consists of a median nerve communicating with a small ganglion placed a little way in front of the brain (Beddard).

The antero-lateral lobes described above are not figured by Vejdovsky (1884) in his drawing of the brain of *Tubifex rivulorum*, and he definitely states that the brain in this region is devoid of processes or lobes. He figures, however, postero-lateral lobes from which well-developed muscles pass to the body wall, and these lobes can be clearly seen (Pl. I, fig. 1, *ps.l.*), but I have never been able to find the muscles. Beddard described, in his definition of this species, a median, smaller, posterior lobe corresponding to the anterior one; but this I have never been able to see. It is particularly easy to see the outline of the brain just at this point, as the dorsal vessel runs under it here, and the red colour of the blood in this vessel causes the brain to show sharply outlined against it. In addition to the lobes already mentioned, there are yet two more to be noted, and these are very important, as it is from them that the peripharyngeal connectives arise. They are situated between the antero-lateral lobes and the postero-lateral lobes, and are slightly more ventral in position than either of these. They pass obliquely outwards, downwards and backwards, and finally bend sharply inwards, terminating in the nervous bands known as peripharyngeal connectives which finally unite; in the median line ventrally, with the first ganglion of the nerve cord known as the sub-pharyngeal ganglion (Pl. II, fig. 8 *sp.g.*).

Cerebral Nerves. I have been able to recognise three pairs of these, two pairs arising from the brain proper and one pair from the peripharyngeal connectives. Of these three pairs of cerebral nerves, one pair arises from the median anterior lobe of the cerebral ganglia. They really originate as one nerve forming a continuation of the median lobe, but this condition only obtains for a short distance, as the single nerve soon divides into two branches which diverge from one another. Both branches, however, pass forwards towards the tip of the prostomium, with which they ultimately become connected. Each nerve branches several times, and gives rise to a large number of extremely fine fibres, which terminate in the sensory cells with which the prostomium is amply provided. It is the presence of these sensory cells which renders the prostomium such an important tactile organ.

The second pair of nerves arising from the cerebral ganglia form continuations of the antero-lateral lobes of the brain, which have already been described. They are short, and pass directly to the lateral walls of the prostomium and first segment. Here they branch, forming a number of fine fibres which spread themselves over and into the body wall of this region. The third pair arise as branches of the peripharyngeal commissure, and pass to the body wall of the first segment, where they also branch (Pl. II, figs. 7 and 8).

The Ventral Nerve Cord originates at that point where the two connectives arising from the cerebral ganglia unite below the gut, having first encircled it. The nerve cord is a ganglionated chain extending from the second segment to the last segment of the body, and it lies freely through all its length in the body cavity. It is surrounded by a connective tissue sheath, from which, at intervals, branches arise and pass to the body wall. By this means the nerve cord is kept in position in the body cavity. It is necessary to exercise care in identifying nerves, for these branches of the connective tissue sheath closely resemble the nerves in appearance, though not, of course, in structure.

The cord is also enclosed in a muscular sheath, which, however, does not completely encircle it, but is confined principally to the dorsal surface. The sheath is very delicate, and is composed of a few muscle fibres placed longitudinally. There is a single ganglion situated near the posterior border of each segment, and these are connected into a continuous chain by a series of connectives which unite adjacent ganglia. In the anterior segments the ganglion and connective are about equal in length, the connective, if anything, being a little longer. More posteriorly, however, where the segments become shorter, the proportions of these two parts of the nerve cord also change. The connectives become shorter and shorter, but the ganglia change very little in size, so that, finally, the connectives are extremely short, and the ganglion occupies almost the whole length of the segment. But throughout the length of the nerve cord the two parts are sharply marked off from one another. This is very clearly seen if one examines the living worm, but when the nerve cord is seen in section the difference between the two is even more apparent. The connective is of about the same diameter throughout its length, but the diameter of the ganglion varies in different parts. In most cases it can clearly be divided into three lobes placed end to end and separated from one another by constrictions. Of these lobes, the first is the largest, while the other two are of nearly the same size, the posterior one being very slightly smaller.

The ventral nerve cord gives off branches or peripheral nerves in each segment. There has been a considerable difference of opinion amongst earlier authorities as to the number of these branches in each segment. Vejdovsky (1884) figures no fewer than five pairs; D'Udekem (1855) states that there are three pairs, but Nasse (1882) was only able to find two. After careful examination of the living worm and sections, I have come to the conclusion that there are three pairs of these lateral nerves in each segment. Their places of origin from the nerve cord are approximately the same in all the segments. There is always one pair arising from the connective and two pairs from the ganglion. The first pair comes off from the connective just behind the septum, while of the two pairs originating from the ganglion one springs from the most anterior of the three lobes, just at that point where the connective merges into the ganglion. The third pair is to be found more posteriorly, and usually originates at or near the constriction which separates the first and second lobes of the ganglion.

These three pairs of nerves all have a similar distribution. They are true lateral nerves, and do not, as in some forms, *e.g.*, most of the Lumbriculidae, arise from the mid-ventral line as a single nerve, and after entering the body wall divide into two branches. They arise, on the contrary, from the lateral part of the cord, but slightly nearer the ventral than the dorsal surface. They pass out at right angles to it, and extend for some distance in the body cavity before plunging into the body wall.

HISTOLOGY OF THE NERVOUS SYSTEM.

In order to get a clear idea of the histological details of the structure of the brain and nerve cord, it is necessary to examine transverse and longitudinal sections of these organs. If the sections are appropriately preserved and stained, the various elements become fairly well differentiated. The two outermost coverings of the nerve cord have already been mentioned, but in sections it can be seen that both the connective tissue sheath and the muscular layer are extremely delicate. The most conspicuous part of the former are the nuclei of the connective tissue cells, and, indeed, in places it is very difficult to identify any other structural details. The nuclei are oval in shape, and the cells to which they belong are considerably flattened. The muscular layer has already been sufficiently described.

The brain is composed of both nerve cells and nerve fibres, the cells being disposed dorsally and laterally, while the fibres occupy the ventral and central parts of the brain. The nerve cells, in section, appear as almost rounded bodies with clearly marked rounded nuclei. The nerve fibres are embedded in a transparent matrix, and when cut transversely give the brain, in the region in which they are, a finely granular appearance.

The nerve cord, as already mentioned, can be divided into connectives and ganglia, the ganglia being represented on the surface of the cord as swellings between the connectives. In sections it can be seen that the difference in size of the cord in different parts is due to the presence or absence of nerve cells. The connectives are formed only of nerve fibres, whereas the ganglia possess in addition a large number of nerve cells, their lateral and ventral position being different from that which they occupy in the brain (Pl. VII, fig. 46, *n.c.; f.*). The mass of nerve fibres, particularly in the ganglia, is divided up into different regions by the interposition between the nerve fibres of a delicate fibrous layer, which appears to be a continuation of the connective tissue sheath which completely surrounds the cord (fig. 46).

As in most Oligochaeta, giant fibres are to be seen in the nerve cord throughout its length. These occupy the same position in the cord as do those of Lumbricus, that is, they lie close to its dorsal surface. The number of fibres present, however, varies in different parts of the body. Anteriorly, just behind the brain and for several segments, the cord contains only one giant fibre, and this lies in the mid-dorsal line. More posteriorly there are three giant fibres, the middle one being the largest. They are still situated dorsally, and lie side by side.

The structure and function, and the history of our knowledge, of the giant fibres in certain Polychaet worms, especially *Halla parthenopcia*, has been dealt with in great detail by Ashworth (1908), and it therefore seems unnecessary to refer to the subject in detail here. It may, however, be advisable to include a brief account of the structure of a giant fibre as described by Ashworth, as follows:—

Each giant fibre consists of a sheath composed of interlacing glia fibres of different diameters and embedded in a finely granular protoplasm. The centre of each fibre is occupied by a bundle of fibrillae, also embedded in a matrix known as the interfibrillar substance. The number of these fibrillae in a fibre differs in different Annelids. The space which is always present between the bundle of fibrillae and the sheath of the fibre is filled with a semi-fluid perifibrillar substance, which is colourless, hyaline and contains very fine granules.

In my preparations of the nerve cord of *Tubifex rivulorum* only the sheath of the giant fibres has been clearly marked, the fibrillae and the perifibrillar substance not staining at all well, thus giving the fibres the appearance of empty tubes. This is due to the extreme difficulty which is always experienced in attempting to differentiate clearly the fibrillae of the giant fibres. All ordinary methods of preserving and staining the material, with which I was familiar, were unsuccessful, and as Ashworth's paper did not come into my hands until this work was ready for publication, I have not yet had an opportunity of testing whether his methods of preserving and staining *Halla parthenopeia* are equally successful in the case of *Tubifex rivulorum.*

THE EXCRETORY ORGANS.

The excretory organs or nephridia consist of a system of paired tubes, which are present in most segments of the body. They are absent from a few of the anterior segments, but as in most aquatic Oligochaeta they begin well before the genital segments, from which, however, they are absent. I have been able to trace these nephridia as far forward as segment 7, but not in front of that. Behind the genital segments they are to be found through all the remaining segments of the body.

The nephridia are coiled tubes occupying two segments, and, typically, two only, but in a few cases I have seen the coils of a nephridium of one segment lying in the segment behind, but this must be considered as an abnormal condition. Each nephridium is provided with an internal and an external aperture, the former opening into the body cavity, the latter to the exterior. The nephridia lie nearer the ventral surface of the body than the dorsal, and are situated one on either side of the ventral vessel and very close to it. In the posterior segments of the body certain of the coils of the tube appear to be very intimately connected with this vessel, so that even though the movements of the worm may be extremely violent, these tubes do not become displaced.

Nasse (1882), in his work on the family Tubificidae, described the presence of branches of the ventral vessel which arise near the nephridia and pass into close connection with these organs. That is, he claims that there are special blood vessels conveying blood from the ventral vessel to the nephridia, in which organs, presumably, the blood is purified. Nasse does not explain how the blood is returned to the main circulation, but he suggests, at any rate, an arrangement of the vessels and nephridia which is closely allied to that found in higher Oligochaeta and typically in Lumbricus. Vejdovsky (1884) contradicts this, and definitely states that there is no connection between the ventral vessel and the nephridia. I am disposed to agree with Vejdovsky that there are no special branches passing from the ventral vessel to the nephridia, but at the same time we ought not to lose sight of the fact that, as has already been stated, the nephridia are always in close connection with the ventral vessel. This suggests the possibility that the excretory products which collect in the blood during its passage round the body are passed out from it, while in the ventral vessel, to the nephridia, the walls of which are very thin and would form no barrier to the diffusion through them of these waste products.

Since the nephridium occupies two segments, it follows that the tube must pierce the intervening septum. We may, therefore, divide the whole nephridium into pre-septal and post-septal portions. The pre-septal part consists of the nephrostome, or funnel, and a very short, uncoiled portion of the nephridial tube. By far the greater part of the nephridium, therefore, lies behind the septum, and consists of a much coiled tube which can be divided into certain regions according to the structure of its walls. This tube terminates in the external aperture or nephridiopore, which is situated at the apex of an enlarged vesicle forming the terminal portion of the nephridial tube. It is possible by careful teasing out of part of the intestinal region of the living worm to separate portions, at any rate, of the nephridium. It is extremely difficult, however, to obtain a good view of the nephrostome and nephridiopore by this means. In some cases the nephridium can be examined *in situ* if the body wall be sufficiently transparent, and in this way the cilia can often be seen in motion. If the tube be removed from the body, the cilia very quickly cease to move, and it is impossible then to decide whether any particular portion of the tube is ciliated or not (Pl. VI, fig. 43).

The nephrostome is small and very simple in structure. Its diameter in the widest place is only slightly greater than that of the nephridial tube. Its lumen is a little larger than that of the tube, and its walls are somewhat thicker, making it funnel-shaped. It is composed of a very few cells, the nuclei of which are large, round and nucleolated (Pl. VII, fig. 47). The inner borders of the cells, those abutting on the lumen, are ciliated, the cilia being long and pointing chiefly in one direction, namely, from the free end of the funnel down the tube. Some of the cilia, however, fringe the free edge of the funnel, and these are particularly long and very active. By their rapid movements they create a current in the direction of the funnel, into which the excretory products are drawn. The pre-septal part of the tube is extremely short and uncoiled, and its cavity is directly continuous with that of the funnel. Its walls are thin, and the nuclei of the cells are flattened in a direction parallel to that of the tube. The tube is ciliated in this part.

The post-septal portion of the nephridium can be sub-divided into three regions:—(1) A delicate, much-coiled tube with extremely thin walls. (2) This passes into a slightly thicker walled tube of a yellowish colour, and decked with specially modified peritoneal cells. (3) This again passes into a somewhat thinner walled tube, which is covered with specialised peritoneal cells for part of its length, and which finally opens into a small vesicle communicating with the exterior at the nephridiopore (Pl. VI, fig. 43, *t*.).

(1) The first portion of the tube has thin, transparent walls, and is profusely coiled, the coils being quite irregular in their arrangement and forming no definite loops such as are so characteristic of the nephridia of Lumbricus and other types. Its cavity is intracellular and ciliated throughout. It is invested by an extremely delicate peritoneal covering, the cells of which are much flattened and possess oval nuclei.

The first portion of the tube is separated from the second by a curious structure, which, as far as I can tell, has never been described by any other observer as occurring in Tubifex. It has been figured by Eisen (1885), however, in the nephridia of *Spirosperma ferox*. He calls it an ampulla, and this name will be used for the corresponding structure as found in Tubifex. Eisen describes the ampulla as an enlargement of the tube of the nephridium, but he does not attempt to give any account of its structure. He does not even venture to express an opinion as to whether it is a permanent or only a temporary structure. Beddard (1895) in his monograph refers to the ampulla in his account of the characters of the family Tubificidae, as occurring in Limnodrilus, *Spirosperma ferox* and many earthworms. He gives, however, no account of its structure.

In Tubifex the ampulla is undoubtedly a constant organ, for I have never failed to find it in any of the specimens that I have examined, whether in sections or by teasing out the nephridium from the living worm. Further, it is present in the nephridium of all segments from the anterior to the posterior end of the body. The ampulla always occurs between the first portion of the nephridial tube and the second, which is characterised by walls of a yellowish colour. The ampulla is usually pear-shaped, although in a few cases it is somewhat more circular in outline. The first part of the tube opens directly into it at its broader end, while the second portion of the tube leaves it at the opposite or pointed end (Pl. VII, fig. 48). The structure of the ampulla is always the same. It has the form of a swollen bladder bounded by a delicate wall composed of a single layer of cells with prominent oval nuclei. The junction of the first part of the tube with the bladder is marked by a circlet of specially large rounded cells, with prominent nuclei, which by their arrangement form a sort of collar round the tube. In the cavity of each ampulla there is, as a rule, a brownish, granular mass of irregular shape lying somewhat nearer to its broader end (Pl. VII, fig. 48, *gr. m.*). In many cases this mass appears to have no connection with the wall of the bladder, but in others I have been able to see exceedingly fine processes extending from it to the wall. In an ampulla removed from the living worm this mass appears to be solid, but in section it is seen to be hollow, its cavity being continuous with that of the first part of the nephridial tube. It is very difficult to decide whether this central canal of the granular mass ends blindly, or whether it communicates with the cavity of the ampulla. I am inclined to think that the latter is the case. There is no suggestion of branching of this canal, nor the formation of fine nephridial tubules within the mass itself. In fact, the latter seems to be composed of a large number of inert granules which are affected by neither killing reagents, preserving fluids nor stains. As already mentioned, the nephridial tube is ciliated throughout, but just at the point where it enters the ampulla it is provided internally with cilia which are particularly long and which project into the ampulla, or more accurately into the canal of the granular mass

(Pl. VII, fig. 48, *ci.*[1]). If one liberates an ampulla with a portion of the tube from the living worm, these cilia exhibit lively movements even for some time after those in the nephridial tubes have become motionless. The wall of the ampulla is not ciliated.

(2) The second portion of the nephridial tube opens out from the pointed end of the ampulla, and directly after its origin it is bent back sharply so that it runs for a short distance parallel to the ampulla and the first part of the tube. This part of the tube is characterised by the fact that its walls are somewhat thicker than those of the first part, that they are yellowish in colour, and the cells of which they are formed are very granular. The tube is not much coiled, but is bent on itself two or three times to form well-marked loops which lie approximately parallel to each other and appear to be bound together in a common investing membrane. For the first part of its length this tube is provided with a layer of peritoneum, composed of flattened cells similar to those investing the first part of the tube. These gradually give place to specially modified cells known as vesicular peritoneal cells (Pl. VI, fig. 44, *v. pt.*). These are large, rounded, bladder-like cells with very thin walls. These cells are particularly well seen in the fresh material, but are liable to undergo considerable shrinkage during the processes of killing and fixing. When examined in the living condition, these cells may be clear and devoid of any special solid contents, or they may be filled with minute brownish granules which exhibit active molecular movements even after the cell has been dislodged from the wall of the nephridium.

(3) The third part of the tube is ciliated, is of somewhat wider calibre than the first part, and has thin walls. The proximal portion is covered with vesicular peritoneal

cells similar to those described above, but distally the peritoneum is again composed of flattened cells. The distal end of this tube expands rather suddenly to form a small, contractile vesicle, which opens to the exterior at the nephridiopore. The tube is ciliated to its distal end, and a tuft of specially long cilia projects into the vesicle. The latter is somewhat pear-shaped when fully expanded, narrowing considerably as it approaches the nephridiopore, which is situated on the ventral surface of the body, a short distance in front of the ventral setae on either side.

REPRODUCTIVE ORGANS.

Tubifex rivulorum is hermaphrodite, the ova and spermatozoa maturing at the same time. The worms exhibit fully-developed reproductive organs during the autumn and early winter, that is, from October to December. The first cocoons are laid about the beginning of November, and can be found in large numbers in the mud for the next two months, after which time their formation ceases. The mature worms show well marked differences in external appearance from those in which the reproductive organs are not well developed, especially in the anterior segments. The sexual organs occupy segments 9-16 or 17 in quite mature worms, and these segments are considerably swollen and of a dull whitish colour, due mainly to the large size of the sperm sac and to the large ova which are present. During May, June and July it is comparatively common to find worms which present somewhat this appearance, and it would be easy to make an error and suppose that such worms were mature if they were not more minutely examined. Further investigation shows that this whiteness and opacity are due to the presence of parasites belonging to the species *Urospora saenuridis*, which will be described later.

The reproductive system is a complex affair, as is usually the case in hermaphrodite forms, and it will be well, first of all, to enumerate the various organs which assist in the process of reproduction and then enter into their structure and arrangement in detail afterwards. First, then, there are the *ovaries* (Pl. I, fig. 2, *ov.*) and *testes* in which the ova and spermatozoa respectively undergo the earlier stages of their development. They are not, however, permitted to come to maturity in these organs, but are transferred quite soon to special sacs known as the *egg sacs* (Pl. I, fig. 3, *ovs.*) and *sperm sacs* (Pl. I, fig. 2, *sp. s.*), which, at first, occupy only one segment each, but in the mature worm may extend through several segments. When the ova and spermatozoa are fully developed, they are transferred to the cocoon by means of special ducts, which are provided with external apertures perforating the body wall. These ducts are known as the *oviducts* (female) and the *sperm ducts* or *vasa deferentia* (male) (Pl. I, fig. 2, *v. d.*). The spermatozoa do not reach the cocoon directly, by means of the vasa deferentia, but are transferred, during copulation, to special organs set apart for their reception. These organs are known as the *spermathecae* (Pl. I, fig. 2, *sp.*). The terminal portion of each vas deferens is dilated to form an elongated chamber known as the *spermiducal gland* (Pl. I, fig. 2, *at.*), which opens to the exterior by the *penis*, a chitinous organ, capable of protrusion (Pl. I, fig. 2, *pe.*). The penis aids in the transference of the sperm from the sperm sac of one worm to the spermathecae of the other during copulation. In connection with the spermiducal gland, and formed as a proliferation of some of its cells, is an irregularly-shaped mass known as the *prostate gland*. The cells of this gland secrete a cementing substance which is passed into the spermatheca with the sperm, and is used for moulding the spermatozoa into a solid mass, of characteristic shape, known as a *spermatophore*. The position which these organs occupy in the body is constant, and will be noticed in the description of the various organs.

I. THE GONADS.

Both ovaries and testes are present in the same worm, there being only a single pair of each. As is usual in the Oligochaeta, the testes lie in front of the ovaries, and in Tubifex they are situated in adjacent segments, the testes in segment 10 and the ovaries in segment 11. In their relative segments they occupy exactly the same position in relation to the other organs present in that segment; that is, they lie one on either side of the intestine and slightly nearer the ventral than the dorsal surface of the body.

Not only do they correspond in their position in the segment, but, in the young condition at any rate, they are exactly similar in appearance and structure. Both ovaries and testes are derived from peritoneal epithelium, and appear in the earliest stages of their development as small masses of a few undifferentiated cells forming the germinal epithelium. In such a condition as this it is only by noticing the segment which they occupy that one can distinguish between the gonads. They are attached to the septum forming the anterior boundary of the segment which they occupy and hang freely into the body cavity. At a little later stage, but when the cells of the germinal epithelium are still undifferentiated, the shape of the mature gonad is indicated. They are both somewhat pear-shaped, the broader end being attached to the septum (Pl. I, fig. 2, *ov.*). In a mature worm, however, the ovaries often attain so great a size that they are somewhat bent round in the segment and the original shape is lost: this is also partly due to the presence of the oldest ova at one side of the ovary. The ovaries persist throughout the reproductive season, and only attain their full size when the rest of the reproductive organs are developed.

If, however, in a mature worm one seeks for the testes, one will not be able to find them. This is due to the fact that they have been completely enclosed in the sperm sac, which is of large size and in which the spermatozoa complete their development. It is necessary, therefore, to examine much younger worms in order to find the testes. In fact, they are quite well developed in those individuals in which there is no trace of any other part of the reproductive system except the ovaries. The testes develop somewhat earlier than the ovaries, and, therefore, in a young worm it will be easy to identify them without verifying their position, as they will be larger than the ovaries. When the testis has attained its full size it consists of a mass of rounded cells with clearly marked nuclei, but without any specially characteristic features. These may be called the spermatogonia, or sperm mother cells, and here the development of the spermatozoa in the testis ceases. The spermatogonia must be transferred to the sperm sac before further development can take place.

Many of the earlier writers confused the testis with the sperm sac or with part of it. D'Udekem (1855), for example, speaks of the testis as occupying segment 8 where it appears as an unpaired organ below the intestine and having the form of a voluminous gland, greyish in colour. What he has described as the testis is really a portion of the sperm sac, for the latter in a mature worm not only extends backwards through several segments, but may pass forwards in front of that segment which originally contained the testis. McIntosh (1871), too, has confused the sperm sac with the testis in stating that the testis of one side remains in segment 9, while its fellow extends back as far as segment 16.

The ovaries are still small when the testes have attained their full size, but while the latter soon disappear the ovaries gradually increase in size until they occupy a large proportion of segment 11. In a fully developed ovary it is easy to recognise ova in several stages of development.

II. DEVELOPMENT AND STRUCTURE OF THE SPERMATOZOA.

The germinal epithelium in the testis gives rise by ordinary cell division to a number of spermatogonia, which, at an early stage in their development, are separated from the testis and pass into the sperm sac, which, at first, is a simple, undivided sac. The spermatogonia, when they leave the testis, are uninucleate, but very soon the nucleus divides several times, and, as its division is not at once accompanied by division of the cytoplasm, each spermatogonium or sperm morula, as it is called, becomes multinucleate (Pl. V, figs. 28 and 29). Calkins (1895A), who has described in detail the spermatogenesis of Lumbricus, states that the spermatogonia, while still in the testis, are multinucleate. Careful examination of many preparations of the spermatogonia of Tubifex has led me to decide that in this form, at any rate, the spermatogonia only become multinucleate on leaving the testis.

The nuclei of the sperm morula, which, when first formed, are scattered irregularly through the cell, gradually become arranged more regularly around the periphery of the cell, the central portion of which has no nucleus and is, therefore, entirely cytoplasmic in nature. The cytoplasm surrounding the nuclei at the periphery of the sperm morula exhibits slight cleavage marks around each nucleus, and these cleavages deepen until the nuclei are completely constricted off from one another, while still remaining in connection with the cytoplasmic mass occupying the centre of the morula, which remains undivided throughout and is known as the sperm blastophore (Pl. V, figs. 30 and 31). During this protoplasmic cleavage the sperm morula or sperm polyplast increases considerably in size, and the nuclei are large, rounded, and possess nucleoli. Each of the elements making up the fully formed sperm morula is called a spermatocyte, and these are at first large and comparatively few in number (Pl. V, fig. 31). The spermatocytes are arranged extremely regularly around the sperm blastophore, but this arrangement can only be fully appreciated when the sperm morula is viewed in section (Pl. V, fig. 32).

The nucleus of each of the spermatocytes now undergoes karyokinetic division, probably twice, and the cells then divide so that the number of elements comprising a sperm morula in its later stages of development is very much increased. The cells thus formed are much smaller than the spermatocytes, as there is no appreciable increase in the size of the whole morula at this stage (Pl. V, fig. 33). The cells resulting from the division of the spermatocytes are known as spermatids, and from these the mature spermatozoa are derived by a simple metamorphosis. The spermatids are at first rounded in shape, and the nucleus forms the greater part of the cell, the cytoplasm being much reduced in quantity. Gradually, however, the spermatids become oval in shape. The nucleus of the spermatid then elongates considerably to form the filiform head of the spermatozoon so characteristic of the group (Pl. V, fig. 34). The middle piece of the spermatozoon is not very conspicuous, but appears as a direct continuation of the head. The distal extremity of the spermatid now becomes much drawn out, forming a long tail which is, in the normal spermatozoon, considerably longer and thinner than the head. It stains but faintly with nuclear stains, as it is composed of cytoplasm only, derived in all probability from the delicate layer of protoplasm surrounding the nucleus of the spermatid. At first, the fully formed spermatozoa are arranged very regularly around the blastophore, as regularly, in fact, as were the spermatids from which they were derived, each one with the anterior end of the head buried in the substance of the blastophore. The tails of the spermatozoa are at first quite straight, and while still in this condition become motile (Pl. V, fig. 35). When the spermatozoon is quite matured and ready to leave the blastophore, the tail usually exhibits a simple coil at the free end. The spermatozoa now become much less regularly arranged on the blastophore and by degrees they separate completely from it. When they are set free they remain for a time in the cavities of the sperm sac, but finally they find their way to the ciliated funnel of the vas deferens, around which, in the mature worm, they congregate in great numbers.

In most of the individuals I have examined, I have found developing in the sperm sac, side by side with the normal spermatozoa, other structures which resemble them to a certain extent, but which can easily be distinguished from them. At first I was inclined to look upon these structures as parasites, but a closer examination of their development has led me to suggest that two kinds of spermatozoa are present. On this understanding the same terminology will be adopted in the following description as was used above in describing the development of the normal spermatozoa.

The central blastophore from which the spermatocytes are constricted off is larger, and more irregular in outline, than that on which the normal spermatozoa are formed. The spermatocytes themselves, too, are less regularly arranged, often tending to be formed in groups of four or five together (Pl. V, fig. 36). The nuclei of the spermatocytes stain very deeply. The changes taking place during metamorphosis can be easily followed. The spermatocytes, which are at first rounded, become oval in shape, the outer free end being considerably more pointed than the opposite end, which is embedded in the blastophore (Pl. V, fig. 37). The spermatids formed by division of the spermatocytes are much larger and less numerous than those developed from a normal sperm polyplast, and their arrangement on the blastophore is as irregular as that of the spermatocytes from which they are derived (Pl. VI, fig. 38). In the earlier stage of metamorphosis there is no semblance of a true tail, but when the head has lost its oval form and becomes much elongated, the tail is gradually differentiated. At first it is quite short, but it increases in length as the head of the spermatozoon becomes fully developed, and in the final stages the tail is as long as, or a little longer than, the head (Pl. VI, fig. 39). The mature spermatozoa are quite irregularly arranged, but they tend to lie together on the blastophore in bundles of five or six, just as the spermatocytes have been described as doing, and from it they finally separate. In some cases I have been able to find in the same sperm smear most of the stages in the development of these spermatozoa, but in other cases they seem to occur much more rarely.

At first I tried to incorporate the stages of development described above in my description of the development of the normal spermatozoa, but when I was able to get such a complete series of each I gave up the attempt. I now suggest that this worm possesses dimorphic spermatozoa, a condition which has been described as occurring in Paludina amongst the Mollusca and in certain Amphibia, Birds and Mammals. In Paludina the two kinds of spermatozoa are different in shape, the normal one being divisible into a spirally coiled head and an extremely long tail. The larger kind is vermiform, and bears a tuft of cilia at one end. In Birds and Amphibia the two kinds of spermatozoa are of the same form, but differ in size, the smaller one being functional.

In *Tubifex rivulorum* both kinds of spermatozoa are elongated structures, and each is divisible into head, middle piece, and tail, but the proportions of these parts to each other in the two forms are rather different (Pl. VI, fig. 40). The head of the normal spermatozoon is small, forming about one-sixth of the total length (Pl. VI, fig. 40A); it is oval in shape with a sharply pointed anterior extremity, and measures about 4μ in length. It is composed almost entirely of nuclear substance, there being such a small quantity of cytoplasm present as to be almost negligible. The middle piece, which is shorter than the head, lies behind that structure, and gives attachment to the tail. It is composed of cytoplasm and stains only slightly with nuclear stains, such as haematoxylin. The tail is very long and delicate, and is also composed of cytoplasm. The other spermatozoa are much larger, and the head forms about half the whole structure. The head is elongated, but less sharply pointed at the anterior end than is that of the normal spermatozoon (Pl. VI, fig. 40B). The middle piece is less distinctly marked off from the head than in the first case, and the tail is straight throughout.

III. THE SPERM-SAC.

The sperm-sac is developed, as in all Oligochaeta, for the reception of the spermatogonia, which are early removed from the testis and transferred to this organ. In it the mature spermatozoa are formed.

In a worm in which the reproductive organs as a whole are immature, the sperm-sac can be seen in its simplest condition. It is an unpaired structure, and arises as a pouch-like outgrowth of the septum between segments 10 and 11. This outgrowth is directed backwards and projects into segment 11. The cavity thus formed is at first quite simple, and is bounded by a layer of flattened peritoneal epithelium which is derived from the tissue of the septum. As the number of spermatozoa increases, the sperm-sac also enlarges, and it soon becomes too big for the segment in which it developed, and projects still more posteriorly into segment 12. In a mature worm the number of spermatozoa is very large, and these are present in the sperm-sac in all stages of development. The sperm-sac increases tremendously in size, extending further and further back until it may occupy as many as seven or eight segments (Pl. I, figs. 2 and 3, *sp. s.*). It is the presence of this large sperm-sac which gives the mature worm a swollen and opaque appearance in the region of the reproductive segments. It is very common also to find that the sperm-sac has encroached upon the segments in front of that occupied by the testis, and segment 9 at least, and occasionally segment 8, also enclose a portion of the sac. The development of this sac both anteriorly and posteriorly to the segment in which it first appears may be due to simple pressure exerted by the developing spermatozoa.

It has already been said that the sac arises as a simple outgrowth of the septum between segments 10 and 11. As it increases in size, however, its structure becomes more complicated. The cavity becomes divided up into a series of much smaller spaces by the growth inwards of its walls. These small spaces remain in communication with one another, and are filled with spermatozoa in all stages of development. It is difficult to believe that the tremendous number of sperm morulae present in the sac have all been derived from the small and inconspicuous testes. Some writers have suggested that the peritoneal epithelium lining these coelomic spaces of the sperm-sac is capable of forming germinal tissue. If all the spermatozoa were derived from the spermatogonia of the testes which are transferred, as they are formed, to the sperm-sac, one would expect to find the youngest sperm morulae nearest the segment in which the testes lie. But this is not the case, for it is common to find spermatogonia and fully developed spermatozoa lying side by side in any part of the sperm-sac, and there is no suggestion of regularity in their arrangement. This irregular arrangement is what one would expect if the epithelium of any part of the sperm-sac were capable of forming germinal tissue.

Throughout the life of the worm the ciliated funnel continues to hang freely into the cavity of segment 10, and never becomes enclosed in the sperm-sac as is the case in Lumbricus. It does not seem quite clear, perhaps, at first sight, how the mature spermatozoa from the sperm-sac reach the funnel. If, however, we bear in mind the development of the sperm-sac, there is little difficulty in understanding this. The sperm-sac extends forwards into segment 9, and backwards as far as segment 16, in both cases ending blindly. There remains, however, an opening from the sperm-sac into segment 10, from which it was derived as simple, pouch-like outgrowths of the anterior and posterior septa, respectively. Although the structure of the sperm-sac becomes much more complicated as the worm matures, the sac retains its connection with segment 10, and so the spermatozoa can pass into the coelom, and thence to the surface of the expanded funnel of the vas deferens which lies in the same segment.

IV. THE SPERM DUCTS OR VASA DEFERENTIA.

The spermatozoa when mature are conveyed to the exterior by special ducts known as the sperm ducts or vasa deferentia. As is always the case in the Oligochaeta, the number of these ducts corresponds to the number of the testes, therefore in *Tubifex rivulorum* there is a single pair. Each duct consists of a much coiled tube with its origin in segment 10—the segment which contains the testes; and its external aperture in segment 11. The whole duct, however, does not lie in these two segments—its length is so great and it exhibits such complex coiling that it extends posteriorly through several segments, sometimes reaching as far back as segment 15.

For the purpose of description we can divide the sperm duct into the following regions:—(1) The ciliated funnel (Pl. I, fig. 2, *ci. f.*), (2) the coiled tube (Pl. I, fig. 2, *v.d.* 1, *v.d.* 2), (3) the spermiducal gland with the prostate (Pl. I, fig. 2, *at.*), and (4) the penis (Pl. I, fig. 2, *pe.*).

These can all be studied in detail by means of transverse and longitudinal sections, but it is interesting and instructive to liberate the sperm duct from the body in the living condition. This is possible by appropriate and careful teasing out of the reproductive segments of a mature worm. During this teasing-out process, the duct can be recognised as a small, whitish, shining mass, formed of a number of coils, which, with care, can be unravelled on the slide.

It is comparatively easy to get the tube with the spermiducal gland and penis still attached, but the structure of the penis cannot be seen well by this means, the only feature which is clearly brought out being the chitinous nature of the penis sheath. It is a much more delicate operation, however, to get the extreme anterior portion of the tubular part with the ciliated funnel still attached. The difficulty is due to the fact that the funnel is situated in a different segment from that occupied by the tube, and the latter is usually broken off at the septum. If one is fortunate enough to get the funnel also, the shape of the latter can be fairly clearly seen, and its appearance will be described later. Although this method of investigation is invaluable for obtaining a correct idea of the relation of the various parts of the sperm-duct to one another, it is of no use for histological details.

1. **The Ciliated Funnels** of the sperm ducts are situated in the segment in front of that which contains the tubular part of the duct, that is, they lie in segment 10, or the segment that contains the testes, and they open directly into the body cavity. When seen in the living condition the funnel has the form of a flat, plate-like expansion, but when sections are examined it will be seen that its shape and appearance vary a good

deal in worms of different ages. In a very young worm in which both testes and ovaries are present, but before the development, to any great extent, of the sperm sac, the ciliated funnel is visible. It is the first part of the vas deferens to be formed, and appears as a local proliferation of the cells of the septum between segments 10 and 11. It rests at first directly on the septum, and it is only at a later stage, after the formation of the tube itself, that it hangs freely into the body cavity. Even at such an early stage as this, it is slightly hollowed out, with its convex side attached to the septum and its concavity opening into segment 10. As the vasa deferentia develop the ciliated funnels increase in size, lose their direct connection with the septum 10/11, and take up their normal position in the segment, being situated one on either side of the intestine and a little ventral in position. Each funnel forms a markedly cup-shaped expansion of the anterior end of the vas deferens. It has a circular outline in transverse section, and opens into the body cavity by a wide aperture (Pl. IV, fig. 18B).

The funnel is composed of a single layer of cells which, when seen in section, are quadrangular in shape, those nearer the centre of the funnel being squarish in outline, while those nearer the edge are somewhat longer than broad (Pl. IV, fig. 17, *ct. ep.*). They are all provided with large nuclei, those in the squarish cells being almost spherical, while the others are oval in shape, but all possess a very distinct nucleolus. This epithelium is ciliated, the cilia being very conspicuous both in stained preparations and in the living specimen. In the latter, the cilia do not seem to be disposed equally over the inner surface of the funnel, but rather to be confined to certain tracts. There is nothing in the sections, however, to suggest this arrangement, and it is very likely that the cilia are not all equally active at the same time, but that some cease entirely to vibrate for a time, and then resume their action again. Outside the ciliated epithelium is a single layer of very much flattened peritoneal cells, which is continuous with the peritoneum, completely investing the rest of the vas deferens. This layer is extremely delicate, but is rendered more conspicuous by the somewhat swollen, oval nuclei which the cells contain (Pl. IV, fig. 17, *pt.*).

The ciliated funnel of a mature worm has a very characteristic appearance. It is no longer cup-shaped, but the edge is so sharply recurved towards the vas deferens that the funnel becomes much more shallow and is almost turned inside out (Pl. IV, fig. 17). The surface of the funnel which bears the cilia is thus exposed as fully as possible. There is not the least doubt that this interesting change in the shape of the funnel is intimately connected with the transference of mature spermatozoa from the sperm sac to the vas deferens. But it is difficult to say whether this change in shape is completed before the spermatozoa actually reach the funnel, in order to expose as much surface as possible for their reception, or whether it is due entirely to the pressure exerted by the enormous number of spermatozoa which congregate upon it. In a mature worm the whole of the ciliated surface of the funnel is covered with a dense mass of spermatozoa, not arranged irregularly, but with their heads entangled amongst the cilia of the funnel, and their tails lying parallel to the cilia (Pl. IV, fig. 17, *sm. t.*). At first sight, the funnel appears to be provided with enormously long cilia, but a closer examination of the stained preparations reveals the true state of things. Just outside the boundary of the cells composing the funnel is a narrow zone which is clearly differentiated from the rest of the apparent cilia. This differentiation is due to the staining properties of this region, for the stain which is absorbed is a nuclear stain such as haematoxylin or borax carmine, whereas the cilia stain only with a plasma stain. This deeply-stained zone just outside the cells of the funnel is caused by the presence of the heads of the spermatozoa, which always stain heavily with a nuclear dye (Pl. IV, fig. 17, *sm. h.*). In many of the preparations which I examined the cilia of the funnel were not visible at all owing to the large number of spermatozoa present, but in a few cases the spermatozoa have slightly shrunk away from the funnel, and in these the true cilia can be seen clearly. Their length is not more than one-third or one-fourth that of the spermatozoon tails. It seems to me that we have here an example of the phenomenon of stereotropism, so well known already in the case of spermatozoa, which appear to be always attracted by any surface. If one examines a funnel in the living condition one often sees a very large number of spermatozoa hovering around and on the funnel, and it is very possible that in the processes of killing and fixing the worm they are maintained in this position.

2. **The Coiled Tube.** The ciliated funnel opens into the tubular part of the sperm-duct, just in front of the septum between segments 10 and 11. The tube, therefore, perforates the septum. It belongs typically to segment 11, but owing to its great length it extends posteriorly through several segments, although its external aperture remains in segment 11. If the tube be examined under the microscope directly it has been removed from the body, it will be seen as a long, delicate, transparent structure with a very clearly marked cavity. If one watches it for a short time it will be seen that the

cavity of one part of the tube is surrounded on all sides by a wall of equal thickness. This part is nearer to the ciliated funnel, and its walls are thin. On the other hand, the part nearer to the spermiducal gland has much thicker walls, and, moreover, these walls contract a little and the cavity of the tube is thrown out of position so that it no longer lies centrally, but takes on the form of a very open spiral, the whole tube meanwhile becoming slightly shorter. If transverse sections of the sperm duct were cut after its removal from the body, the canal would be seen to be lying nearer the outer wall, first on one side, then on the other. In sections of the sperm duct cut when it is in position in the body, however, the canal lies centrally for all its length, which seems to indicate that the spiral curve of the canal in the first case was due to contraction on its removal from the body. Beddard states in his Monograph on the Oligochaeta that the coiled tube in all the members of the Tubificidae is ciliated throughout, but this statement is not entirely correct. In the immature form of *Tubifex rivulorum* cilia are present throughout the whole length of the duct, but the coiled tube of the mature worm can be divided into two parts, one ciliated, the other non-ciliated. The former lies nearer to and in connection with the ciliated funnel, while the latter opens into the spermiducal gland. These ciliated and non-ciliated parts of the tube can be easily recognised, both in the living condition and in sections. We will first confine our attention to the vas deferens of the mature worm, though it will be necessary later on to refer to the immature form for the purpose of comparison.

(*a*) The ciliated portion of the tube occupies about half its total length, and follows immediately upon the ciliated funnel (Pl. I, fig. 2, *v.d.* 1). Ciliary action does not occur by any means regularly in this part, for it is often completely in abeyance, but the cilia are so long that they can be easily seen, even when they are motionless.

In the wall of the ciliated portion of the tube we can recognise the following layers:—(1) Ciliated epithelium, (2) Muscular layer, and (3) Peritoneum.

The ciliated epithelium forms the innermost layer of the wall of the duct and is composed of a large number of flat, annular or ring-like cells piled very regularly on one another. Each cell is perforated in the centre by a large, rounded lumen, about 42 μ in diameter, the wall around it being comparatively thin. A continuous tube is formed by the regular arrangement of the cells and by the fact that the cavity is pierced in exactly the same position in all the cells (Pl. IV, fig. 20A). Each cell possesses a single, elongated, somewhat spindle-shaped nucleus which may extend almost half way round the cell (Pl. IV, fig. 19A). It is pointed at both ends and somewhat broader near the middle, where it exhibits a well-marked, rounded nucleolus. The inner edge of each cell is plentifully provided with cilia which are rather longer than the radius of the cavity, and have a somewhat spiral arrangement (Pl. IV, fig. 19A, *ci*). The cells themselves appear to be embedded in a structureless matrix which stains with plasma stains and forms an extremely delicate layer right round this part of the duct. The centre of the tube is usually occupied by a dense mass of spermatozoa, which in the living condition can be seen travelling down the tube. The muscular layer is but feebly developed in this part of the tube. It consists of a single layer of longitudinal muscle fibres which are disposed at equal distances around the tube (Pl. IV, fig. 20A; *l.m.*). They do not pass straight down the wall of the tube, but have a somewhat spiral arrangement. The peritoneum, which completely invests the tube, is composed of a single layer of very much flattened cells with rather large, oval, nucleolated nuclei (Pl. IV, fig. 20, *pt.*).

(*b*) The non-ciliated portion. The ciliated part of the tube gives place gradually to the second part, which is characterised by the absence of cilia and the presence of a much thicker wall. Consequently, this part of the canal has a diameter almost twice as great as that of the first part (Pl. I, fig. 2, *v.d.* 2.). The lumen of the tube is of exactly the same diameter throughout, so its increase in size is due entirely to the greater thickness of its wall.

The epithelial cells which form the innermost layer of the wall of this second part of the tube have the same general form as those described above. That is, they are ring-shaped, piled one on top of the other extremely regularly, and each one is perforated in the centre by a rounded lumen. Each cell has its greatest thickness around the lumen of the tube, and tapers off considerably at its outer edge (Pl. V, fig. 21). The nuclei of these cells resemble very closely those of the first part of the tube, and are situated near the inner edges of the cells (Pl. IV, fig. 19B). The greater thickness of the wall of this part of the tube is due to a considerable increase in the quantity of the matrix in which the cells are embedded, and by which they are surrounded (Pl. V, fig. 21, *ma.*). This matrix still stains only with plasma stains, but it is no longer structureless, for it is characterised by the formation in it of a large number of exceedingly fine fibrillae. These are not visible in the fresh material, but are rendered very distinct by the action upon them of fixing reagents and by the use of appropriate stains.

They are arranged very regularly, originating at the inner edge of the cell and radiating out to the periphery. These fibrillae are so numerous that there is but little of the normal cytoplasm remaining. Owing to the large number of these fibrillae which are present, and to the consequent disappearance of the cytoplasm of the cells, the cell boundaries become very indistinct, and are barely recognisable. The nuclei, however, persist in their original position, and indicate, sufficiently clearly, what was the arrangement of the cells (Pl. IV, fig. 20B).

In the mature worm these cells are not ciliated, but the inner edge of each is considerably thickened and strengthened by the deposition of a secretion resembling cuticle in appearance and staining reactions. Since the inner edge of each cell is thus thickened, the secretion has the form of a series of circular bands arranged extremely regularly throughout the length of the tube (Pl. IV, fig. 20B, *an.r.*). It is extremely difficult to decide what is the exact nature of the substance composing these rings. Its position in relation to the cell and to the lumen of the duct suggests a chitinous or cuticular secretion, but there is no doubt that it is capable of contraction, which suggests that it is muscular in nature. These circular bands convert this part of the vas deferens into a much more solid structure than it would otherwise be, and also help to keep the lumen open. Outside the epithelial layer is a single layer of longitudinal muscle fibres arranged similarly to those of the first part of the tube. They are continuous, at the end of the vas deferens, with those which surround the spermiducal gland. A peritoneal covering invests the vas deferens in this part also, and its cells are flattened, forming a single layer, and provided with oval nuclei.

The change from the narrower to the wider part of the vas deferens is a gradual one, and this transitional part of the tube is worth further examination. As has been already noticed, the lumen remains of the same size throughout. The wall, however, thickens gradually. The cells of which it is composed become somewhat larger, and are a little further apart from one another, but their boundaries are quite distinct (Pl. V, fig. 21). The matrix increases in quantity, it is developed between adjacent cells and also in a thick layer outside them. The fibrillae are quite distinct, and very numerous even in this region. For a short distance the cells of the second part of the tube are ciliated, but there are no annular rings. As soon as the rings appear the cilia are lost, and at this stage also the cell boundaries become less distinct.

The vas deferens of quite a young worm is ciliated throughout, and the wall of the second part of the tube is only very slightly thicker than that of the first. That is, the matrix is only present in small quantities at first, and there are but slight indications of the annular rings.

It seems strange that so little notice has been taken of the complicated structure of the vas deferens in this worm, and, possibly, in many others. Beddard, as well as stating that the tube is ciliated throughout, adds in another place that the wall in Oligochaeta as a whole is usually composed of an inner ciliated epithelium and an outer peritoneal covering. He seems to consider it a very exceptional condition for there to be any muscular elements at all, and he quotes Eudrilus (an earthworm) as his only example. Nasse (1882) seems to have been the first and only observer to note the essential points in the structure of the vas deferens of Tubifex, but his description gives one the impression that he was uncertain on some points, such as the actual form of the cells, whether they were annular or only spindle-shaped, and whether an external peritoneal covering is present or not. In a paper recently published in the *Zoologischer Anzeiger*, Dr. Bohumil Cejka (1913) gives a short account of the structure of *Litorea krumbachi*, in which he deals with the vas deferens. From his description of the histological structure of this tube, I am inclined to think he has seen something of the structure which I have attempted to describe. He says, for instance, that two regions are recognisable. In a transverse section of the first part of the duct he describes the tube as circular and the lumen as being provided with strong cilia. At the periphery he has seen black fibrils, which he believes play an important part in contraction. The second part of the tube, he says, is not ciliated, and has thicker walls which are composed entirely of gland cells—a structure which is quite different from that just described for *Tubifex rivulorum*.

3. **The Spermiducal Gland with the Prostate.** This gland has the form of an elongated, somewhat pear-shaped and curved sac, into the broader end of which opens the second part of the vas deferens, while the narrower end communicates with the exterior by the penis, which will be described later. The cavity of the spermiducal gland is a direct continuation of the cavity of the vas deferens, and is also continuous with that of the penis (Pl. VII, fig. 45). We may, therefore, consider the gland and penis as being modified portions of the sperm duct. Attached to the gland at one side, and near its swollen extremity, is a glandular, irregularly-shaped, lobate mass forming what is known as the prostate (Pl. VII, fig. 45, *pr.*). The cells of which the prostate is composed are large and pear-shaped, with prominent rounded nuclei situated usually in the swollen part of the cell. The narrower part of each cell forms its

duct, and in sections these are all seen to converge towards one point, that point being where the prostate is in communication with the spermiducal gland. At this point, the muscular and peritoneal layers of the wall of the gland are interrupted so that the cells of the prostate and those of the innermost layer of the gland are intimately connected with one another. In fact, it has been said by those observers, *e.g.*, Vejdovsky, who have studied the development of the spermiducal gland and the prostate, that the latter is simply formed as a proliferation of the cells of the lining epithelium of the gland.

The wall of the spermiducal gland is composed of the following layers:—(*a*) An inner epithelium, (*b*) A muscular layer, and (*c*) Peritoneum.

Beddard states that the lining epithelium of the spermiducal gland in Limnodrilus is ciliated, and this suggests the possibility that a similar condition obtains in the other members of the family Tubificidæ. Vejdovsky certainly believed it to be ciliated in Tubifex, as he states that the prostate is derived from cells of the ciliated lining epithelium. In the mature worm, at any rate, I have never been able to identify cilia in the gland, and as the second portion of the vas deferens is not ciliated this is not surprising. The cells of which this epithelium is composed differ a good deal in structure and appearance at different stages of their development. In a young worm, whose reproductive organs are all developed but not yet matured, the lining epithelium of the spermiducal gland is composed of a single layer of rather low cells, cubical in shape, with oval nuclei which are large and conspicuous and situated near the outer end of the cell. The cell is filled with a dense, granular cytoplasm, which stains deeply with nuclear stains such as borax carmine. Just at the junction of the prostate with the gland, this single layer of cells gives place to a mass of irregularly shaped, somewhat elongated cells which encroach a good deal upon the cavity of the gland in this region. In a mature worm the appearance of these cells is very different. They become much enlarged, so that they tend to obliterate the cavity of the gland. Their outlines become less distinct, and the nuclei, which remain near the outer end of the cells, are very difficult to identify. This increase in the size of the cells is accompanied by a change in their contents. The cytoplasm becomes less granular and very vacuolated, and its appearance suggests the presence of some secretion which stains readily with "plasma" stains, such as picro-indigo-carmine (Pl. VII, fig. 45). Outside the epithelium is a layer of longitudinal muscle fibres placed somewhat obliquely, and outside this again a delicate peritoneal covering of flattened cells.

4. **The Penis.** The terminal portion of the spermiducal gland is modified to form a protrusible penis which opens to the exterior on the ventral surface of segment 11. The structure of the penis is somewhat complicated. Its cavity is continuous with that of the gland, and it is surrounded by a penis sheath, which, when the penis is retracted, is double, the outer layer being continuous with the epidermis of the body wall (Pl. VII, fig. 45, *pe.s.*). We may, therefore, speak of the outer and inner penial sheaths, both consisting of a single layer of epithelial cells, somewhat cubical in shape and provided with conspicuous, rounded, nucleolated nuclei (Pl. V, fig. 22, *pe.s.* and *pe.s.*^{1}). The cuticular secretion, which forms a delicate layer outside the cells of the epidermis, is continued over the cells of the penial sheath, and in the retracted condition of the penis may completely fill the space between the outer and inner penial sheaths. The

outer sheath is provided with muscles externally, these being disposed mainly in two ways (Pl. V, fig. 22). There is a comparatively delicate layer of circular muscles completely investing the sheath, and, from this layer, oblique muscles pass, in a somewhat irregular fashion, to the body wall. Certain of these fibres branch, and the branches anastomose with the longitudinal muscles of the body wall.

The cavity of the penis proper is surrounded by a single layer of elongated cells (Pl. VII, fig. 45, *le.*) which encroach considerably on the lumen. Near the apex of the penis they are so large that they almost obliterate the lumen altogether.

V. The Development and Structure of the Ova.

The ovary of a young worm consists of a solid mass of undifferentiated cells, of which those which are nearest to the septum to which the ovary is attached are the youngest. The nuclei of these cells are often to be seen undergoing mitotic division, and by this means the number of the cells making up the ovary is increased. The first formed cells are gradually pushed further away from the septum, and these give rise to potential ova or oogonia, which can be recognised by the large size of their nuclei as compared with the amount of cytoplasm surrounding them.

Vejdovsky suggests that in such a form as Rhynchelmis the oocytes are at first amoeboid, and that their subsequent rapid increase in size is due to the ingestion of the ova around them. I have never been able to identify amoeboid oocytes in Tubifex, but some sections have revealed what appears to be an equally effective mode of nutrition. The young ovary is, as has already been stated, composed of a solid mass of cells, all of which seem to have equal chances of developing into mature ova. In many cases, however, development only continues in those cells around the periphery of the ovary. The central cells do not develop any further, but gradually lose their individuality, the cell membranes disappear, and the nuclei are absorbed. These changes in the central cells result in the formation of a central, non-nucleated, cytoplasmic core surrounded by developing oocytes. It is interesting to notice that these oocytes are distinctly pear-shaped: the narrower part of the cell being embedded in the central mass, while the nucleus is lodged in the broad part of the cell (Pl. V, fig. 26, *oc.*). Certain of these cells develop much more rapidly than the others, and increase so much in size that they protrude considerably beyond the surface of the ovary, and are only enclosed by a fine membrane composed of a single layer of much-flattened cells. It is very easy to conceive of these oocytes being dislodged from the ovary, and this is what actually does happen, for when they have reached a certain size they are transferred to the egg sac, while still immature, and here they complete their growth.

In the course of my investigations of the living worm, I have several times succeeded in teasing out and freeing from the body the ovary, in a more or less complete condition. In most cases, its appearance very nearly resembles that seen in sections, and although the oocytes in this condition are very transparent, it is possible, by the addition of a little methylene blue, to stain them sufficiently to examine them before disintegration sets in, which, by the way, takes place very rapidly. As a rule, there is no special blood supply to the ovaries. It has already been mentioned that the perivisceral vessels of segments 10, 11 and 12 are much enlarged in the mature worms, and that these vessels give off important branches which are directed backwards over the reproductive organs. There are, however, no special branches from these vessels to the ovaries. In one case I found quite a different state of affairs on teasing out the ovary from the living worm (Pl. V, fig. 25). Only a portion of the ovary was obtained on this occasion, but the oocytes present were situated on all sides of a central "stem" or rod in which a blood vessel was lying—this could easily be identified by the red colour of the blood which was still present in it (Pl. V, fig. 25, *st.*). The oocytes were arranged quite irregularly on the stem. Large and small ones lay side by side, sometimes grouped together into clusters, sometimes occurring singly. Each oocyte was attached to a short branch of the main stem terminating in a tiny swollen head, the oocyte itself being spherical in outline, or nearly so. Opposite to each stalk a short branch was given off from the blood vessel—this branch entered the stalk and terminated in the swollen head already mentioned. It was not possible, of course, to decide what was the connection of this "ovarian vessel" with the rest of the circulatory system, as the ovary was completely isolated. Although I examined many more ovaries I never saw this interesting condition again.

As the oocytes contain no yolk-granules as long as they remain part of the ovary, they are then most suitable for purposes of examination, as the yolk-granules tend to obscure the other structures in the egg (Pl. V, fig. 27). The structure of such an oocyte of Rhynchelmis has been described in detail by Vejdovsky (1884), but the description does not entirely agree with the conditions obtaining in an oocyte of Tubifex, at the same age.

The oocyte is spherical in outline, and surrounded by a delicate investing membrane. The cytoplasm forms a fine network, the density of which is only slightly greater at the periphery than nearer the centre of the cell. In Rhynchelmis, Vejdovsky figures a much denser portion of the cytoplasm around the periphery with a more delicate network in the interior. The nucleus is large and rounded, and at this stage is situated near the centre of the cell, but as the ovum matures it comes to lie nearer the periphery. The nucleus is surrounded by a well-marked nuclear membrane, but I have not been able to identify any perforations through which the nucleoplasm is in communication with the cytoplasm, such as are figured by Vejdovsky as occurring in Rhynchelmis. The nucleus is composed of a large number of chromatin granules embedded in the usual linin network—the linin stains only faintly, but the chromatin stains much more deeply, and, therefore, shows up well in good preparations.

The nucleus contains one or more nucleoli, which appear to have a different structure in different ova. In all cases there is one large nucleolus which is spherical in outline, and which we may describe as the "principal" nucleolus.* In some cases this nucleolus has a vacuolated appearance, showing one or two fairly large vacuoles, in other cases it stains uniformly and appears to be an undifferentiated mass of chromatin, while in yet other ova it has a granular appearance (Pl. V, fig. 27, *p.nul.*). In addition to this principal nucleolus there is usually a number of smaller masses which we may call "accessory" nucleoli.* These are not always present, but in those oocytes in which they do occur their number varies from 2 to 5. These accessory nucleoli very often have no connection with the principal one, but sometimes one large accessory nucleolus is in conjunction with the principal one, forming a compound body. In this case the accessory nucleolus usually stains more deeply with a nuclear stain than does its companion (Pl. V, fig. 27, *ac. nul.*).

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* Compare Wilson's "The Cell in Development and Inheritance," p. 127.

Very soon after the oocyte has been transferred to the egg sac, yolk granules appear in the spaces of the cytoplasmic network, the cytoplasm meanwhile shrinking away from the wall a little. Later on the yolk granules become so numerous that the protoplasm cannot be seen at all. The formation of the granules seems to have no connection with the position of the nucleus, that is, they are scattered irregularly through the cytoplasm. The granules are spherical bodies of comparatively large size, varying from 2 μ to 4 μ in diameter. They stain very deeply with both nuclear stains such as haematoxylin and plasma stains, such as picro-indigo-carmine (Pl. V, fig. 24, *y. gr.*).

It is interesting to notice that maturation of the egg-cell actually begins while the latter is still enclosed in the egg sac, but I have not been able to decide exactly how nearly the process is completed before the egg is extruded, as all the specimens I have obtained which have been undergoing maturation have been at about the same stage. The oldest oocytes within the egg sac often exhibit a fully-formed nuclear spindle, the nuclear membrane having already disappeared (Pl. V, fig. 24). The spindle is somewhat dumbbell-shaped, and exhibits the normal structure. The two centrosomes are far apart from one another, but are connected by a large number of spindle threads. The star or aster (Pl. V, fig. 24, *as.*) is formed by a number of delicate threads which, surrounding the centrosome, radiate out into the adjacent cytoplasm. The chromatin has grouped itself into a number, about 24, of small rounded masses, which are arranged on the equator of the spindle (fig. 24, *chr.*). The nucleus at this time is situated near the periphery of the cell.

VI. THE EGG SAC.

The egg sac is set apart for the reception of immature ova which have become separated from the ovary. In this organ the ova complete their growth and undergo the first stage of their maturation process. The egg sac is an unpaired structure, and arises as a single, pouch-like outgrowth of the septum between segments 11 and 12. It is directed backwards, and in its simplest condition occupies only segment 12. But it soon enlarges considerably, and extends into the segments behind the twelfth. The sperm sac in the course of its development becomes pushed into the egg sac, so that we have here the curious condition of one sac lying actually within the other (Pl. III, fig. 11, *sp.s.*, *ovs.*).

The cavity is, of course, coelomic in origin, and remains undivided throughout, that is, there is no division of its cavity into smaller ones, such as occurs in the sperm sac. The wall of the egg sac is thin and composed of a single layer of flattened peritoneal cells, with no muscular elements at all (Pl. I, fig. 3, *ovs.*).

Egg-cells of different ages can be found in the egg sac, and their position in this organ suggests very forcibly that they have all been derived from the ovary. The youngest oocytes are situated in the most anterior segments: that is, in those nearest the ovary, and many of these have few or no yolk granules. The oocytes, when liberated from the ovary, fall into the cavity of segment 11, in which the ovary lies. As the egg sac is an outgrowth of the posterior septum of this segment, it is a very simple matter for the oocytes to find their way from the segment into the egg sac.

VII. THE OVIDUCTS.

There has been a considerable difference of opinion amongst earlier observers as to the position of the oviducts in most members of the Tubificidæ. Without going into the whole history of the question, we may notice that D'Udekem (1855) believed that in *Tubifex rivulorum* the oviducts were connected with the male ducts, indeed that they actually surrounded the terminal portion of the vasa deferentia. His views were supported by later observers, such as Claparède (1861) and Eisen (1885). Vejdovsky at first was inclined to agree with D'Udekem's description of the relations between these two ducts; but later he changed his opinions, and came to the conclusion that the oviducts are situated between segments 11 and 12. This conclusion was based upon certain experiments which he performed. For example, he kept worms in certain chemical reagents, and observed the extrusion of the eggs between these two segments.

For some time I was unable to identify the oviducts in my sections, but Mr. E. S. Goodrich, F.R.S., was kind enough to lend me some of his slides in which they were shown quite plainly. Since then, on a re-examination of my own preparations, I have been able to distinguish these structures, though much less clearly than in those of Mr. Goodrich—to whom I wish to express my indebtedness.

There can be no doubt that, while the oviducts are small and comparatively inconspicuous, they are normally present, at any rate in the fully mature worm. There is a single pair lying in the intersegmental line 11, 12 (Pl. I, fig. 2, *ovi.*). The duct is very short, and opens internally by a wide, funnel-shaped opening into the coelomic cavity of segment 11, while the external opening, which is small and inconspicuous, lies in the same longitudinal line as the male openings.

VIII. THE SPERMATHECAE.

During copulation the spermatozoa of one worm are transferred to the other, and stored in special organs known as the spermathecae (Pl. I, fig. 2, *sp.*). In *Tubifex rivulorum* there is one pair of these organs situated in the same segment as the testes, viz., in segment 10. Their general form is best seen by liberating them from the living worm. This can easily be done if a mature worm be placed on a slide and a cover-glass put upon it. The slightest pressure on the cover-glass is usually sufficient to rupture the body wall in the region of the reproductive segments, which are much distended owing to the large number of spermatozoa and ova developed. If the body wall be ruptured, the spermathecae, which for the greater part of their length lie freely in the body cavity, are usually forced out, but remain attached to the worm at their external apertures (Pl. VII, fig. 49). The ease with which this operation can be performed is due to the fact that the spermathecae are of considerable length and are bent round in the segment. They are also resistant to pressure, to some extent, and as soon as the body wall of the worm is ruptured they spring free. They are visible to the naked eye in this condition as small, pear-shaped, opaque, but glistening bodies, but it is necessary, of course, to examine them under the microscope in order accurately to describe their form. When examined thus, the whole organ can be divided into two regions:—(*a*) a pouch or sac-like portion (Pl. VII, fig. 49, *sp. p*^{1}.) which narrows considerably to form (*b*) the duct opening to the exterior near the ventral setae of segment 10 (Pl. VII, fig. 49, *sp. d.*).

The proportions of these two parts to one another vary considerably according to the condition of the worm—in a mature worm the duct is usually somewhat longer than the pouch, but in the younger condition they are more nearly equal. Typically, the spermatheca lies entirely in segment 10, but about the time of copulation, and certainly when the spermatozoa are to be found in these organs, they enlarge considerably and may be either coiled round in segment 10 or extend into segment 11, or even more posteriorly into segment 12 (Pl. IV, fig. 16, *sp.*). In the latter case there is a sharp bend in the spermatheca, and the duct runs forwards again parallel to the pouch in order to open to the exterior on segment 10. The pouch is quite simple, although it is so voluminous, and is devoid of diverticula; neither has it any connection with the alimentary canal, as is the case in certain Enchytræidae.

The structure of the wall of the spermatheca differs considerably in different parts. The wall of the pouch is thin, but three layers are recognisable. Internally there is a single layer of epithelial cells which seem to be of two kinds. Certain of the cells are squarish in shape and have very large nuclei, which are oval or rounded in shape, and usually include one principal and one or more accessory nucleoli. These cells are probably glandular in nature. Between these are smaller, narrow cells, whose nuclei are much less conspicuous, and which we may term interstitial cells. The wall of the pouch is strengthened by the presence of a few scattered muscle fibres placed parallel to the longitudinal axis of the spermatheca and outside the layer of epithelial cells. Outside this again, the wall is covered by a single layer of flattened peritoneal cells, the nuclei of which are oval in shape and form the most conspicuous part of the cells.

The duct when seen in transverse section is circular in outline, and its wall shows the same layers as are to be seen in the wall of the pouch, but their structure and arrangement are somewhat different (Pl. V, fig. 23). There is a very marked change in the character of the cells forming the inner epithelium. There has been considerable discussion amongst the earlier observers as to whether the epithelium of the duct is ciliated or not in the spermathecae of species belonging to the family Tubificidae. Beddard, although he mentions the views of different authors upon the subject, does not venture any opinion himself. He states in his general account of the spermathecae of the Oligochaeta that ciliation does not, as a rule, occur, but that it has been described in a few forms, for example, Tubifex. As no reference was given, I have been unable definitely to trace the paper from which he obtained this information. It seems likely, however, that he is referring to a paper by Nasse (1882), which he mentions in his general description of the family Tubificidae. Nasse definitely states that there are cilia in the duct of the spermathecae of Tubifex, "In Ausführungsgange trägt das Epithel eine Cuticula und flimmert." Vejdovsky (1884), careful observer though he was, has denied the presence of cilia, and Stolc in his monograph on the Tubificidae (1888) does not figure them. I am now in a position to confirm the statement made by Nasse, for in sections of the spermathecal duct, both transverse and longitudinal, the cilia are particularly obvious (Pl. V, fig. 23, *ci.*). They are not visible when one examines the spermatheca in the living condition, for the wall of the duct is rendered opaque by a thick, muscular covering. The epithelium of the duct is composed of a single layer of cells almost columnar in shape, but considerably longer than broad, and with large oval nuclei. The cilia are rather short, shorter considerably than the radius of the cavity of the duct, so that they do not obstruct the passage much.

The muscular layer is much more powerfully developed around the duct than on the pouch. The muscles form a double layer, those on the inside being arranged circularly, while outside this is a very definite sheath of longitudinal muscles. These appear to be continuous with those on the pouch, the circular muscles being interposed between them and the ciliated epithelium. The cells of the peritoneum covering the duct are somewhat different from those investing the pouch. They are no longer flattened, but squarish in outline, and form a rather more conspicuous layer than that of the pouch (Pl. V, fig. 23, *pt.*). The nuclei are usually situated near the middle of the cell, the contents of which are very granular.

The terminal portion of the duct of the spermatheca is protrusible, and when it is protruded the duct narrows considerably near the end and leads to the exterior by a very narrow but straight passage. Near the spermathecal pore (Pl. IV, fig. 16, *sp. p.*) the ciliated epithelium ceases, and that which lines the narrow passage leading to the exterior is similar in structure to that forming the epidermis of the body wall, with which it is directly continuous.

The muscular layer extends to the end of the duct, where the muscles become connected with those of the body wall. When the terminal portion of the duct is retracted, the passage to the exterior is no longer straight, but its wall is folded back in the form of a cone or arrow-head.

IX. THE SPERMATOPHORES.

A spermatophore consists of a large number of spermatozoa cemented together by a substance derived from the prostate, the cells of which open into the spermiducal gland as already described. These spermatophores are comparatively solid and resistant structures, and can always be found in the spermathecae of a mature worm. When properly formed, they have a very characteristic shape and structure, but it is quite common to find ill-formed ones which are not at all typical in shape or structure. This seems to be due to a lack of the cementing substance.

The number of these structures which may be found in one spermatheca varies considerably. I have often found only one, more commonly two or three, and occasionally as many as five or six. They vary, too, very much in size, but, as a rule, the perfectly formed ones are nearly or quite as long as the spermatheca in which they are found. They are arranged quite irregularly in the spermatheca, sometimes lying coiled up entirely in the pouch, and in other cases occupying the duct and whole length of the pouch, and yet lying coiled up in it. Their arrangement can best be seen when the spermathecae are liberated from the living worm, as described above (Pl. VII, fig. 49). They can then be examined within the spermatheca, or, with care, it is possible to liberate them by rupturing the wall of this organ, when those which are coiled up in it break free and may be removed in a perfect condition. They stain well in a very dilute solution of methylene blue, but permanent preparations cannot be made in this way. They can, however, be fixed to the slide, and stained first with borax-carmine and counter-stained with picro-indigo-carmine, when the various parts will be well differentiated, the heads of the spermatozoa staining well with the borax-carmine, and the tails with the picro-indigo-carmine.

The spermatophores are visible to the naked eye, and when first liberated from the spermatheca appear as small, fine, white, glistening bodies, the largest being about 1-2 mm. long. When examined under the microscope they will be seen to have quite a complicated structure, but before describing this it will be well, perhaps, to say a word or two about their formation. During copulation, the spermatozoa, together with the secretion of the gland cells of the prostate, are transferred from one worm to the spermathecae of the other. It seems certain that the moulding, which must take place before the spermatophores attain their final shape, is carried out entirely in the spermatheca, and moreover in one part only of the spermatheca, viz., its duct. The duct leading from the external aperture to the pouch is so narrow, and the quantity of sperm forced in so great, that the mass must necessarily conform very closely to the shape of the duct. And this we find is the case even to the minutest detail. We will first describe the general form and minute structure of the spermatophore, and then show how this agrees with the general shape of the spermathecal duct.

The fully-formed spermatophore is a narrow, much elongated structure, many times longer than broad, and somewhat worm-like, its widest part being near the middle and tapering off at both ends. The anterior end is always simple in structure, but the posterior end may be similar to it or may terminate in a beautifully moulded and curved conical head (Pl. VII, fig. 50). It has been suggested that the presence or absence of a conical head is characteristic of two different species, but this is impossible, as I have found spermatophores with and without the head in the same spermatheca or in the two spermathecae of the same individual. I shall refer to this again later.

The minute structure of these spermatophores has been described by Vejdovsky (1884), and by Lankester (1871, A). When examined in transverse section, the spermatophore is seen to be circular in outline. In the centre is a cavity which we will call the axial cylinder (Pl. VII, figs. 51 and 52, *ax.c.*). It extends from end to end of the spermatophore, and even into the conical head. It is widest in the centre, and tapers off at both ends. This cavity is filled with a substance which, when the spermatophore has just been liberated from the worm, is granular in appearance. This central mass stains deeply with borax-carmine, and in permanent preparations has the appearance of a mass of longitudinal fibres, which usually become somewhat shrunken by the action on them of reagents. Outside the axial cylinder is a very narrow dark band, which stains much more deeply than the substance of the axial cylinder (Pl. VII, figs. 51 and 52, *sm. h.*). For this reason it is very conspicuous. When highly magnified it is seen to consist of a very large number of minute oval masses, which, owing to their staining properties, I conclude are the heads of the spermatozoa. The rest of the spermatophore is made up of a cementing material in which the tails of the spermatozoa are embedded (Pl. VII, figs. 51 and 52, *sm. t.*). As was pointed out both by Vejdovsky and Lankester, these are placed parallel to one another, but obliquely to the axis of the spermatophore. The tails of the spermatozoa appear as striations in the homogeneous substance in which they are embedded, and when viewed from the surface are seen to pass obliquely round from left to right.

Both Vejdovsky and Lankester state that not all the tail is embedded in the cementing material, but that a small portion lies outside, so that the whole spermatophore is surrounded by the free ends of the spermatozoa. The spermatophores which I have examined show a certain amount of variation in this respect. In many cases the tails are undoubtedly partly free from the cementing material, and the free ends are placed at right angles to the longitudinal axis of the spermatophore, and not obliquely to it. In some cases, however, the layer of cementing material is thicker than in others, and when that is so there is no portion of the spermatozoon lying free. Whether this is an abnormal condition, or merely due to the amount of cementing material present, is very difficult to decide.

Lankester's description differs from this in several details. He states that there is a narrow, highly refringent band outside the axial cylinder and another of similar nature outside the layer of cementing material and spermatozoa, between it and the free ends of the tails of the spermatozoa, the latter being in constant vibratile motion. He also says that the whole spermatophore exhibits movement when liberated from the spermatheca into a dilute salt solution. Although I have performed this operation many times, I have never succeeded in persuading the spermatophore to exhibit the active movements which have been described. Further, Lankester believes that the head of the spermatozoon is much elongated, of almost the same length as the tail, and that the head as well as part of the tail is buried in the cementing material towards the outside of the spermatophore. I think there is no doubt that the heads of the spermatozoa lie just outside the axial cylinder, but very close to it, and are confined to the deeply staining zone described above, and that the oblique striations already referred to are caused by the tails of the spermatozoa alone.

It is very evident that the very characteristic form of the spermatophores is due entirely to the shape of the spermathecal duct and its external aperture. The duct is circular in section, so also is the spermatophore. During the rapid movements of the worm it is very natural that the spermatophore should be forced through the duct into the wider pouch by a slightly spiral motion, and this explains the oblique arrangement of the tails. The muscular walls of the duct may also assist in forcing the spermatophore into the pouch. As a rule, the first-formed spermatophores, those which are forced into the pouch soon after or during copulation, are pointed at both ends or without the arrow-shaped head described above. On the other hand, it is very usual to find that the spermatophores which are formed last, that is, those which lie partly in the duct and partly in the pouch, are provided with the conical extremity. There can be little doubt that the arrow-shaped head is moulded in that part of the duct following directly on the external aperture, for the shape of the two are exactly similar when the spermathecal duct is retracted. I conclude, therefore, that the spermatophores, moulded first in the spermatheca, are forced through the duct and into the pouch quite rapidly—partly by pressure from behind exerted by other masses of spermatozoa and cementing material, and partly by the contractions of the worm. At any rate, the process would seem to be such a rapid one that the spermatophore does not remain any length of time in the duct. On the other hand, towards the close of copulation, the formation of the spermatophores would take place more slowly, and the posterior ends of the last one or two could remain in the terminal portion of the spermathecal duct a sufficient time to allow of their being moulded to its shape. It is true that I have never observed the posterior end of the spermatophore lying in this position, but I have found it several times only a short way along the duct and with the conical head perfectly moulded.

X. THE COCOON.

It is common amongst all Oligochaeta for the ova and spermatozoa to be deposited in cocoons, and *Tubifex rivulorum* is no exception to the rule. The worms are sexually mature in the autumn, and the cocoons are first seen in November. They are deposited on the mud in which the worms live, and very soon are completely buried in it. The cocoons are made of a fibrous substance secreted by the glandular cells of the clitellum, and have a characteristic form. They are usually whitish or greyish in colour and semi-transparent, but when viewed with the naked eye they appear opaque, but this is due to the eggs which they contain. They are usually oval in shape, but sometimes become more nearly spherical, and at either end they are drawn out into a short neck through which, when the young worms are ready to hatch out, they emerge (Pl. VII, fig. 54). The neck is filled with a plug of the same substance of which the cocoon is formed, but it is probable that it is of a less resistant nature. The number of eggs which may be present in a cocoon is very variable. Sometimes there is only one, more commonly from four to nine, but occasionally the number is greater, and in a very few cases I have seen as many as thirteen or fourteen. Although I have not been able to find any spermatozoa even in a freshly-laid cocoon, there can be no doubt that in *Tubifex*, as in other Oligochaeta, the spermatophores are passed into the cocoon with the eggs, and are there dissolved to set the spermatozoa free. The rest of the cocoon is filled with a clear, colourless fluid, probably albuminous in nature. It seems to depend largely on the number of eggs in a cocoon as to whether they all develop into embryos or not. I have been able to count as many as nine young worms in a cocoon, all of which hatched out, but never more than that number; so it is probable that, as a rule, all the eggs in a cocoon develop, but when the number is unusually large certain only of them develop, the others breaking down. The young worm when hatched exactly resembles the adult in external form, and, with the exception of the reproductive organs, all the organs of the body are well represented. The newly-hatched worm is about a quarter of an inch long, and consists of 30 to 35 segments. The alimentary canal is complete with the exception of the anus, which does not develop until later. The whole canal contains a large number of yolk granules, the remains of those found in the egg, and there are some also in the coelomic cavity. The dorsal and ventral blood vessels are visible, the former already slightly contractile. The setae are perfectly developed, but small in size, and there are not more than two or three in each bundle in the anterior segments, and one more posteriorly.

PARASITES.

Tubifex rivulorum is a host for several internal parasites, and in addition to these it often has attached to the body wall externally Vorticellae and Fungi.

The Vorticellae are not true parasites, for they all possess an active circlet of cilia at the distal end and a mouth. The worm is simply made use of in this case as a substratum to which the Vorticellid becomes attached. These Protozoa are very abundant on some individuals, and completely absent from others, but, as a rule, when present, they are confined to the posterior segments of the body, those which wave about freely in the water.

The fact that Fungi often attack these worms was recognised by McIntosh (1871), who speaks of Fungi growing on the dis-organised anterior segments, while the posterior ones are in full activity. I have often observed fully active worms which are infected with Fungus growths, and it seems very probable that the Fungus increases so much in quantity that it actually causes the disintegration of the segments which are attacked—the anterior segments are usually affected first, but the growth may spread throughout the entire length of the body. These Fungi appear, as a rule, to attach themselves in or near to the setigerous sacs, for they are usually to be seen emerging from between the two prongs of the sigmoid setae, forming long, delicate filaments, several of which may originate from the same seta-bundle.

The internal parasites may be found in the alimentary canal, sperm sac and body cavity. McIntosh states (1871) that he has found numerous examples of Opalina amongst the sandy mud in the intestinal canal. He gives figures of some of these specimens, which have very diverse forms. These worms were captured from the margin of the River Tay. It is interesting to note that, although I have examined numerous worms from the Thames both in sections and in the living condition, I have never once found Opalina in the alimentary canal. I have been more successful, however, in finding parasites in the sperm sac and body cavity. During the summer months of 1912, when the worm was really immature, several specimens were found which at first sight had the appearance of maturity, for, in the region of the reproductive organs, the body was white, swollen and opaque. When the body wall in this region was punctured, parasites escaped in large numbers. At first they appeared as small, rounded, bodies, white and glistening, each of which proved to be a cyst bounded by a fairly thick wall (Pl. VII, fig. 53 A). If a cover-glass be put on, or the cyst be subjected to slight pressure, the wall bursts and its contents are liberated. These consist of an enormous number of extremely minute spores, each one somewhat awl-shaped, and provided with a caudal filament at one end. At the opposite end is a rounded, clear space, and between the two what may be described as the body of the spore, consisting of granular protoplasm and embedded in it an elongated nucleus, apparently broken into two parts (Pl. VII, fig. 53 B, *nu.*). This parasite was first described by Kolli, and was named by him *Urospora saenuridis.* It is a Gregarine of the sub-order Eugregarinae, and tribe Acephalina.*

McIntosh (1871) described these parasites as awl-shaped bodies, but he did not appreciate their real significance, as he interpreted them "as stages in the development of the spermatozoa." Nasse also saw them (1882), and interpreted them correctly as parasites occurring in the sperm sac. Minchin* mentions another Gregarine parasite (*Synactinomyxon tubificis*) as occurring in the sperm sac of *Tubifex rivulorum,* but I have not been fortunate enough to find it.

The last parasite noticed as occurring in Tubifex during my observation of the worm was Caryophyllaeus, a Cestode belonging to the family Caryophyllacea of the

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* *Vide* "A Treatise on Zoology."—Ed. by E. Ray Lankester. Part I, pp. 194 and 298.

Cestoidea Monozoa. The parasite occupies the coelomic cavity, and has only been found during the summer months, when the reproductive organs are undeveloped. The mature form is to be found in the intestine of certain fishes, and the young in the anterior segments of *Tubifex rivulorum*, usually in segments 10 to 17. Each one is provided at the anterior end with a characteristic mobile organ capable of being thrown into a series of undulating folds. At the opposite end the cylindrical body is produced into a tail provided with three pairs of hooklets.

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EXPLANATION OF PLATES.

LIST OF REFERENCE LETTERS.

a. = Uncinate setae from ventral bundle.

ac. nul. = Accessory nucleolus attached to principal one.

ac. nul^1. = Accessory nucleolus.

am. = Ampulla.

an. l. = Antero-lateral lobe.

an. r. = Annular ring of vas deferens.

ar. h. = "Arrow-head" extremity of spermatophore.

as. = Aster.

at. = Spermiducal gland.

ax. c. = Axial cylinder.

b. = Uncinate setae from dorsal bundle.

bl. = Blastophore.

br. = Brain.

bu. c. = Buccal cavity.

b. v. = Blood vessel.

b. v. i. = Blood vessel in intestinal wall.

b. w. = Body wall.

c. = Pectinate seta.

ca. f. = Caudal filament.

ca. s. = Capilliform setae.

c. c. = Chloragogen cells.

ce. = Cells of vas deferens.

chr. = Chromosomes.

cht. = Chromatin.

ci. = Cilia.

ci. f. = Ciliated funnel.

cl. = Clitellum.

cle. = Cleavage marks.

c. m. = Circular muscles.

co. s. = Connective tissue sheath.

ct. ep. = Ciliated epithelium.

cu. = Cuticle.

cu^1. = Prolongation of cuticle into setigerous follicle.

cy. = Cytoplasm.

cy. m. = Undivided cytoplasmic mass to which oocytes are attached.

d. = Capilliform setae.

d. bl. = Dorsal seta bundle.

d. t. = Dorsal tubes or neurochord.

d. v. = Dorsal vessel.

e. = Subsidiary prongs.

eg. = Eggs.

e. m. = Opening of prostate into spermiducal gland.

epi. = Epispore.

f. = Fibres of nerve cord.

fb. s. = Fibrillar substance.

fl. = Flagellum.

g. c. = Gland cells of epidermis.

gr. m. = Granular mass.

h. = Large blackish-brown granules.

ht. = Heart.

hyp. = Epidermis.

i. = Smaller granules of chloragogen cells.

in. = Intestine.

in. c. = Interstitial cell of epidermis.

in. v. and *in. v*^{1}. = Intestinal vessels.

le. = Large cells surrounding the lumen of the penis.

l. m. = Longitudinal muscles.

m. = Muscles from pharynx to body wall.

ma. = Matrix.

m. f. = Intrafollicular muscles.

mi. = Middle piece.

m. l. = Anterior median lobe.

mo. = Mouth.

m. p. = Parieto-vaginal muscles.

mu. = Muscular layer.

mu. l. = Muscular layer, circular inside, longitudinal outside.

n. = Nerve cord.

n. b. = Nerves to body wall.

n. c. = Nerve cells.
n. cd. = Non-ganglionated part of nerve cord.
n. cd^1. = Ganglionated part of nerve cord.
ne. = Nephridium.
n. f. c. = Nuclei of young follicle cells.
np. = Nephridiopore.
n. pr. = Nerve to prostomium.
ns. = Nephrostome.
n. s. f. = Nuclei of setigerous follicle.
nu. = Nucleus.
nu^1. = Nuclei in mitotic division.
nul. = Nucleolus.
nu. mem. = Nuclear membrane.
nu. sp. = Nuclear spindle.
oa. = Ovum.
ob. m. = Oblique muscles.
oc. = Oocyte (pear-shaped).
oc. y., *oc. y*^{1}. = Young and older oocytes respectively.
oe. = Oesophagus.
ov. = Ovary.
ovi. = Position of oviduct.
ovs. = Ovisac.
pe. = Penis.
pe. s. = Outer penis sheath.
pe. s^1. = Inner penis sheath.
ph. = Pharynx.
p. nul. = Principal nucleolus.
pp. c. = Peripharyngeal commissure.
pr. = Prostate.
pro. = Prostomium.
pr. t. = Preseptal part of nephridial tube.
ps. l. = Postero-lateral lobe.
pt. = Peritoneum.
pv. v. = Perivisceral vessel.
pv. v. 2 = Perivisceral vessel of segment 2.
pv. v. 3 = Perivisceral vessel of segment 3.
r. = Wall of nephrostome.
s. = Setae.
se. = Septum.
se. c. = Secretion of gland cells.
s. gl. = Septal glands.
s. f. = Setigerous follicle.
si. v. = Supra-intestinal vessel.
sm. t. = Tails of spermatozoa.
sm. h. = Heads of spermatozoa.
sp. = Spermatheca.
spc. = Spermatocyte.
sp. d. = Spermathecal duct.
sp. g. = Sub-pharyngeal ganglion.
sph. = Spermatophore.
spm. = Spermatids.
spo. = Spores.
sp. p. = Spermathecal pore.
sp. p^1. = Spermathecal pouch.
sp. s. = Sperm sac.
s. s. = Setigerous sac.
st. = Stalk.
t. = Contractile enlargement of nephridial tube.
te. = Position of the testes in the immature worm before the development of the sperm sac.
u. = Wall of ampulla.
va. ep. = Vacuolated epithelium.
v. bl. = Ventral seta bundle.
v. d. = Vas deferens.
v. d. 1 = Ciliated part of vas deferens.
v. d. 2 = Non-ciliated part of vas deferens.
v. pt. = Vesicular peritoneal cells.
v. v. = Ventral vessel.
w. cy. = Wall of cyst.
x. = Thin-walled coiled part of tube.
y. = Wider part of tube with vesicular peritoneal cells.
y. gr. = Yolk granules.
z. = Thick-walled tube leading to nephridiopore.

PLATE I.

Figs. 1 to 3. Diagrammatic representation of the first eighteen segments of the body with the principal organs in each segment, as they appear in the living worm.

PLATE II.

Fig. 4. Longitudinal section through the prostomium and first six segments of the body to show the anterior part of the alimentary canal and nervous system. × 160.

Fig. 5. Section through one of the dorsal setigerous sacs. × 510.

Fig. 6. Isolated setae from dorsal and ventral bundles. × 300.

Fig. 7. Transverse section through the first segment of the body, showing the buccal cavity and brain. × 200.

Fig. 8. Transverse section through the first segment, showing brain, peripharyngeal connectives and sub-pharyngeal ganglion. × 200.

PLATE III.

Fig. 9. Transverse section through segment 8 in the region of the hearts. × 160.

Fig. 10. Transverse section through segment 11, showing the ovaries and one spermatheca. × 100.

Fig. 11. Transverse section through segment 14, showing the ovisac and sperm sac. × 90.

Fig. 12. Longitudinal section through the body wall of a mature worm in the region of the clitellum. × 520.

Fig. 13. Longitudinal section through the wall of the intestine, showing the details of its structure. × 370.

Fig. 14. Chloragogen cells in the living condition, drawn directly after their separation from the wall of the intestine. The nucleus is not shown, as it is very difficult to see in the fresh cells. × 1000.

Fig. 15. Chloragogen cells: the cells were separated from the wall of the intestine, fixed in Perenyi's fluid and stained with Brazilin on the slide. The irregularity in shape is due to the shrinkage. × 1000.

PLATE IV.

Fig. 16. Longitudinal section through the reproductive segments 10-13 of a mature worm. One of the spermathecae (*sp.*) only is shown cut twice. It is much enlarged and extends into segment 12. The septa between the segments were not visible in the preparation. × 120.

Fig. 17. Longitudinal section through the ciliated funnel of a mature worm covered with spermatozoa, showing its structure and connection with the vas deferens. × 230.

Fig. 18. (A) Longitudinal section through the ciliated funnel of an immature worm. × 250.

(B) Transverse section of the same. × 250.

Fig. 19. (A) Transverse section of ciliated portion of vas deferens. × 800.

(B) Transverse section of non-ciliated portion of vas deferens. × 800.

Fig. 20. (A) Longitudinal section through ciliated portion of vas deferens. × 800.

(B) Longitudinal section through non-ciliated portion of vas deferens. × 800.

PLATE V.

Fig. 21. Longitudinal section of vas deferens. The section shows the transition from the ciliated to the non-ciliated part of the duct. × 800.

Fig. 22. Transverse section of the retracted penis. × 570.

Fig. 23. Transverse section of the spermathecal duct. × 360.

Fig. 24. An oocyte from the ovisac, undergoing maturation. × 160.

Fig. 25. An unusual form of the ovary which was liberated from the body and drawn in the living condition.

Fig. 26. Longitudinal section through a portion of the ovary, showing pear-shaped oocytes. × 480.

Fig. 27. Longitudinal section through a portion of the ovary, showing typical young and older oocytes, the latter just before their separation from the ovary. × 280.

Figs. 28-35. Stages in the development of the normal spermatozoa.

Fig. 28. Uninucleate spermatogonium from testis. × 820.

Fig. 29. Multinucleate spermatogonium from sperm sac. × 1720.

Fig. 30. Early stage in the formation of the spermatosphere, showing the first cleavage of the cytoplasm. × 1720.

Fig. 31. Formation of spermatosphere complete. Surface view of spermatosphere with spermatocytes. × 470.

Fig. 32. Section of spermatosphere with spermatocytes. × 470.

Fig. 33. A rather later stage. × 670.

Fig. 34. Formation of spermatids which are just beginning to elongate. × 610.

Fig. 35. Spermatozoa fully formed, but still attached to the blastophore. × 350.

Figs. 36-40. Stages in the development of "giant" spermatozoa.

Fig. 36. Formation of spermatocytes. × 660.

Fig. 37. Spermatids just beginning to elongate. × 660.

PLATE VI.

Fig. 38. Later stage in the elongation of the spermatids. × 660.

Fig. 39. Immature "giant" spermatozoa. × 660.

Fig. 40. (A) Ordinary form of the spermatozoa. × 1710.

(B) "Giant" form of the spermatozoa. × 780.

Fig. 41. A figure drawn from a living specimen to show the arrangement of the blood vessels in the prostomium and first five segments of the body. The coiling of the perivisceral vessels is slightly simplified. × about 220.

Fig. 42. A figure drawn from a living specimen to show the arrangement of the blood vessels in an intestinal segment. × 180.

Fig. 43. A diagrammatic representation of a complete nephridium.

Fig. 44. A small portion of the nephridial tube covered with vesicular, peritoneal cells—drawn in the living condition.

Plate VII.

Fig. 45. Longitudinal section through the spermiducal gland, prostate and penis. × 110.

Fig. 46. Transverse section of the nerve cord. × 630.

Fig. 47. Longitudinal section of a nephrostome with the preseptal part of the nephridial tube, part of the body wall and septum. × 520.

Fig. 48. Longitudinal section of an ampulla of a nephridium. × 790.

Fig. 49. Spermathecae liberated from the body through a rupture in the body wall caused by pressure. × 50.

Fig. 50. External form of a very large spermatophore. × 55.

Fig. 51. Longitudinal section of part of a spermatophore. × 620.

Fig. 52. Transverse section of a spermatophore. × 620.

Fig. 53. (A) Section of a complete cyst of *Urospora saenuridis*. × 390.

(B) Detailed drawings of two of the spores. × 1900.

Fig. 54. General view of a cocoon with ripe eggs.

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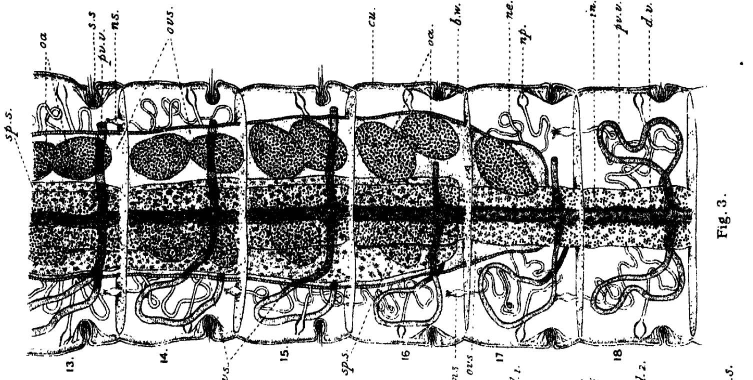

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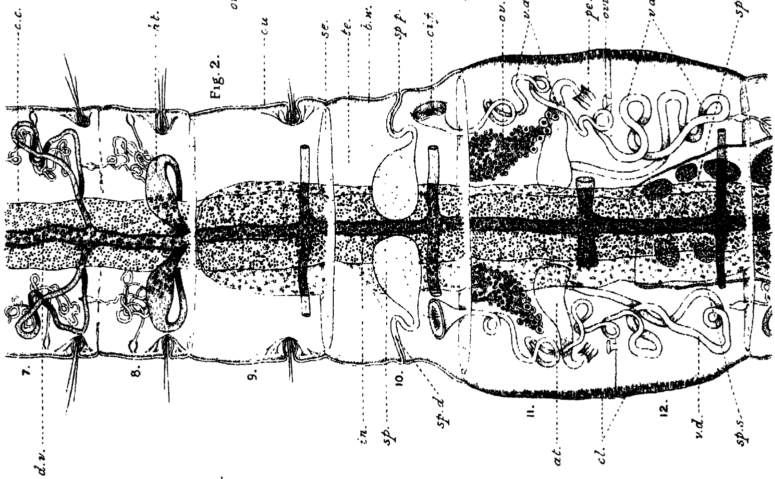

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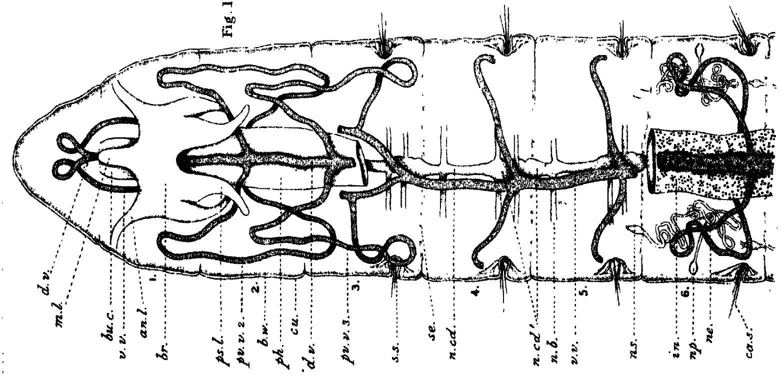

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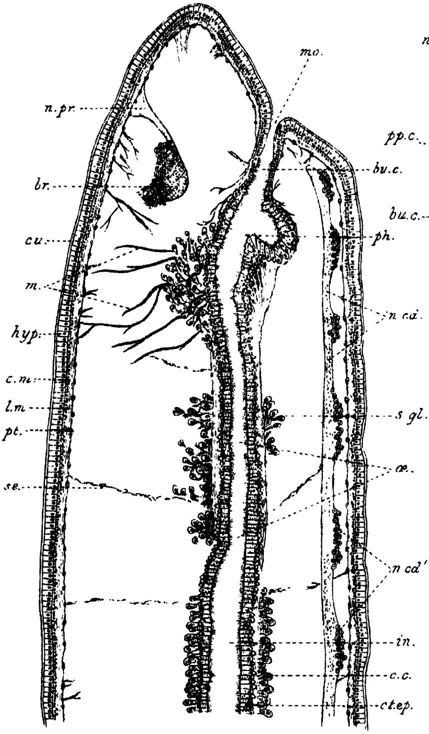

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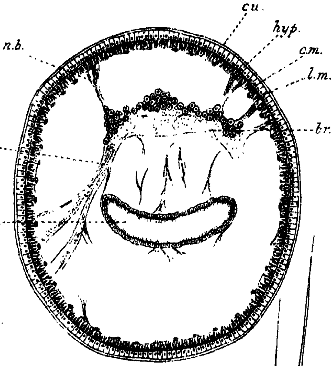

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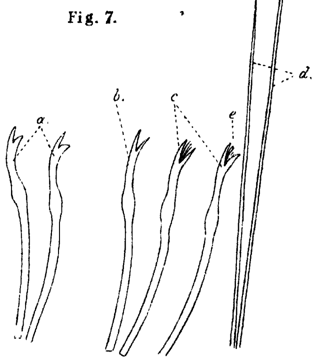

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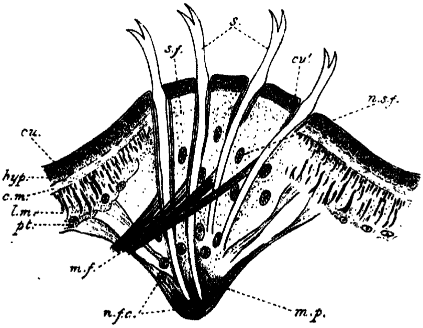

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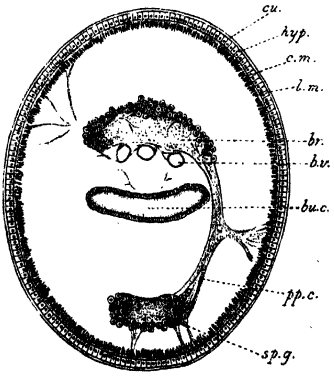

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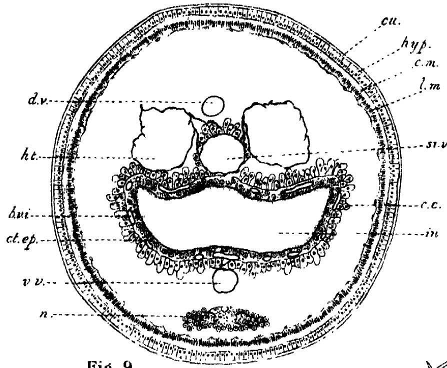

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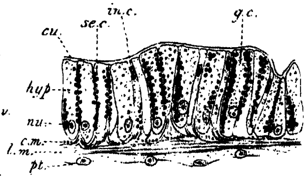

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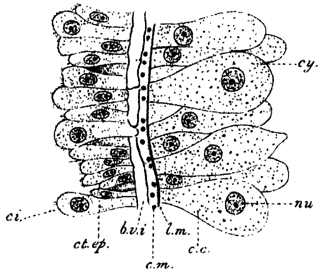

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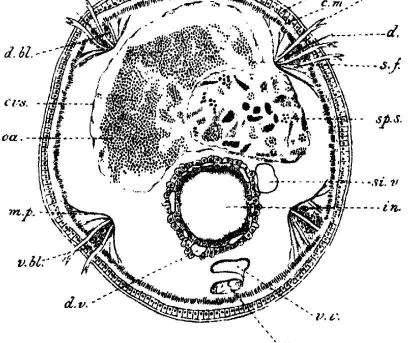

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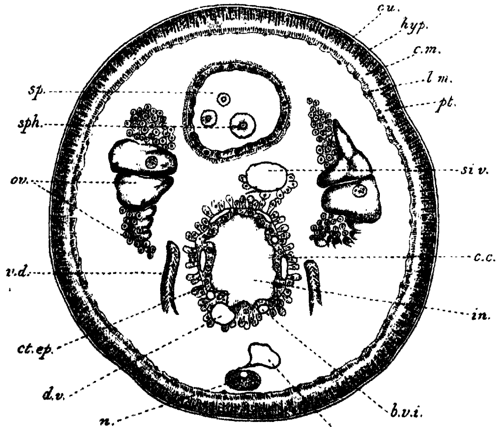

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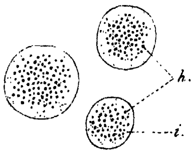

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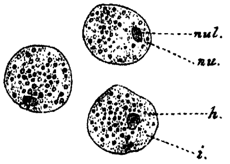

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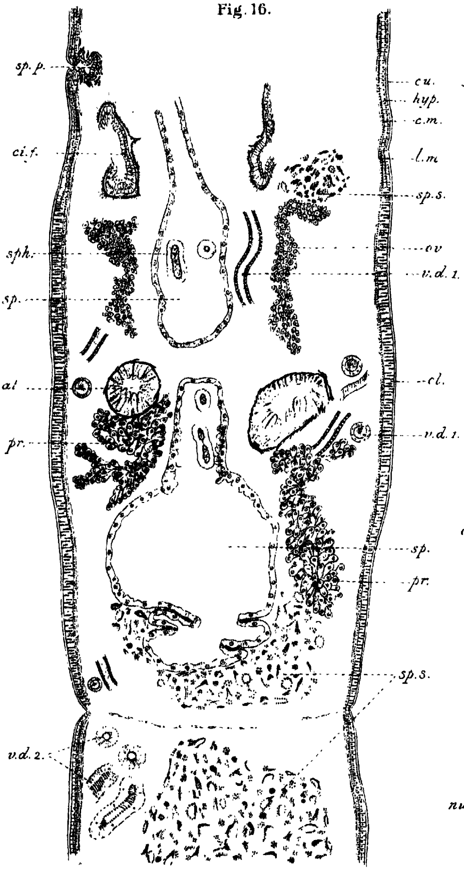

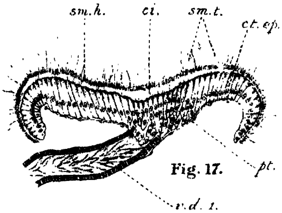

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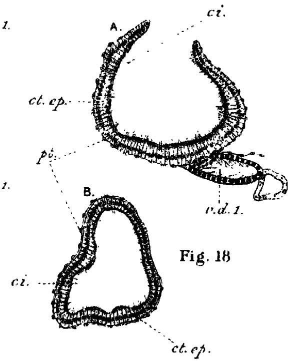

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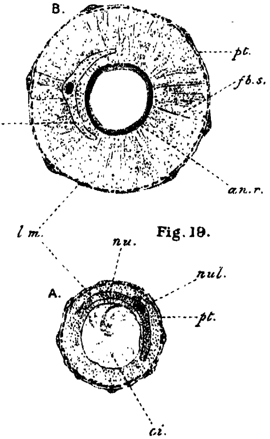

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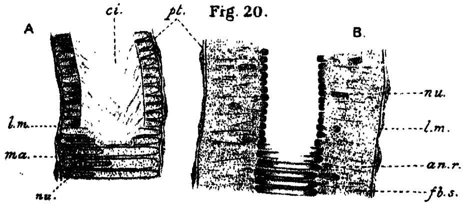

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TUBIFEX.

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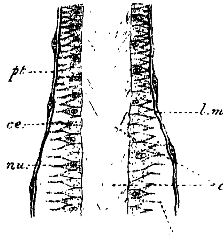

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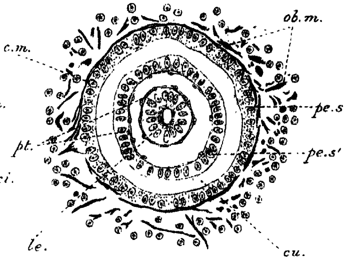

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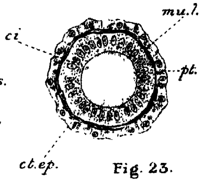

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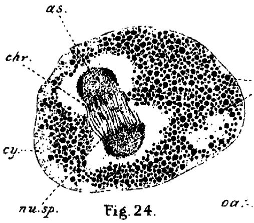

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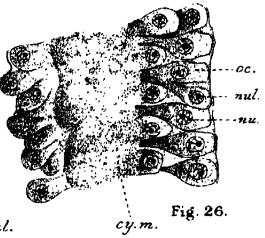

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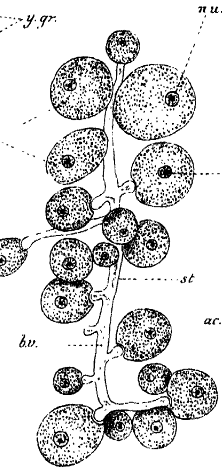

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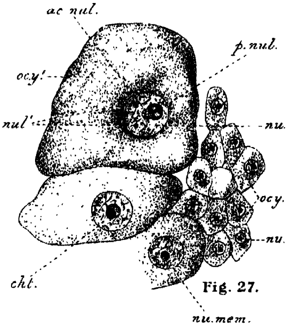

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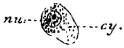

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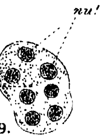

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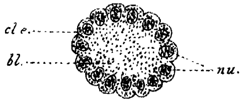

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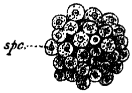

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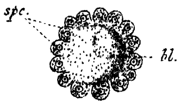

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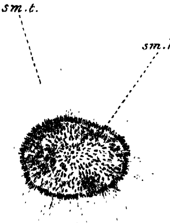

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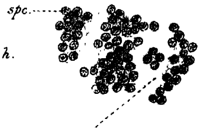

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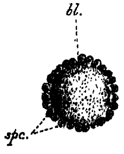

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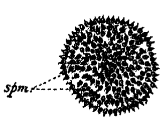

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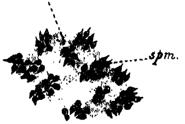
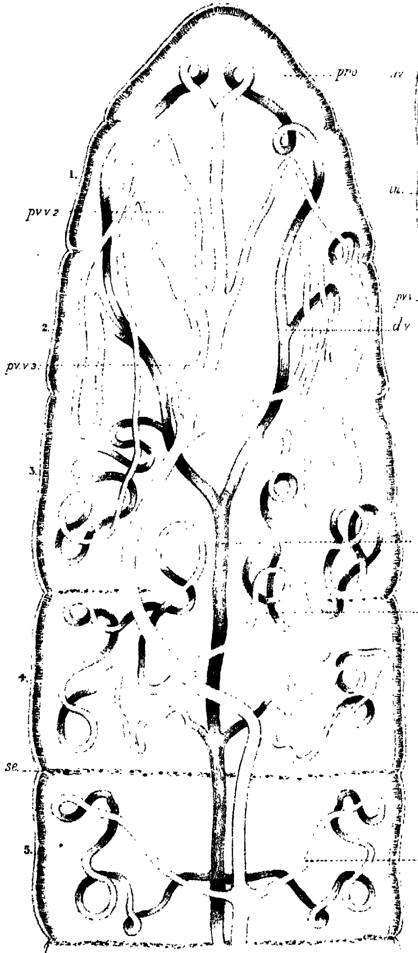


Fig. 37.

G.C.D. del.

TUBIFEX.

McFarlane & Erskine, Lith. Edin.



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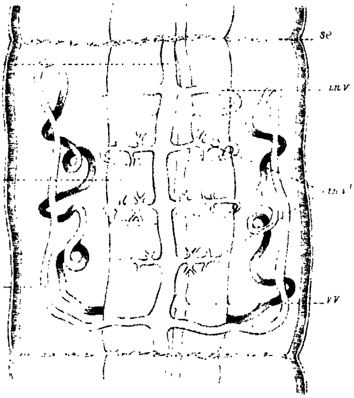

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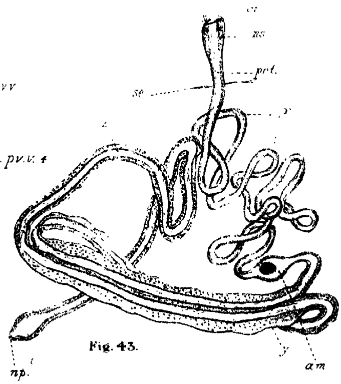

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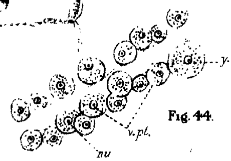

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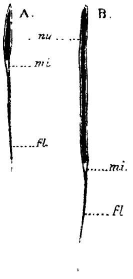

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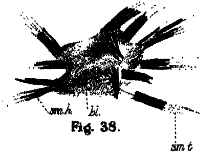

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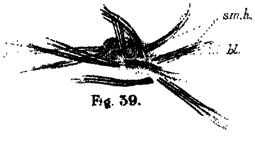
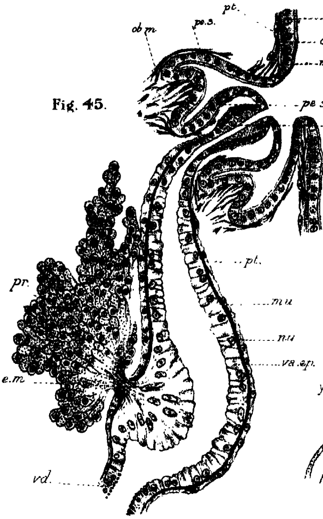


Fig. 39.

G.C.D. del.

TUBIFEX.

M'Farlane & Erskine, Lith. Edin.



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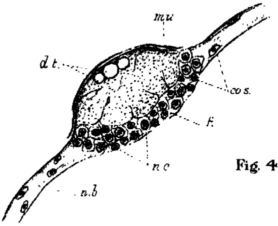

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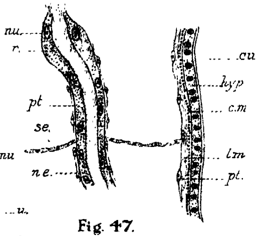

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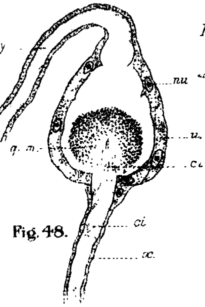

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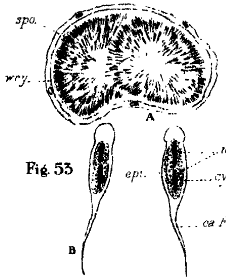
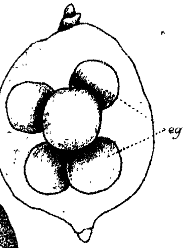


Fig. 53



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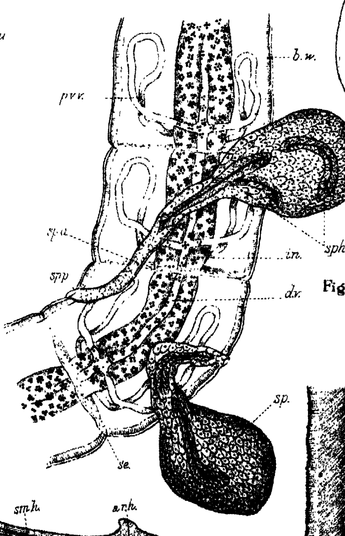

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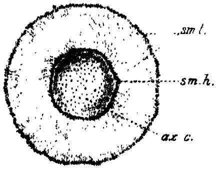

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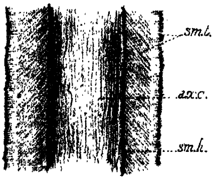

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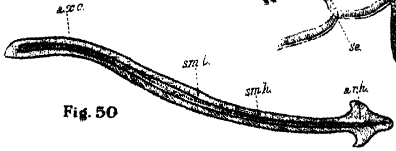

Fig. 50

G.C.D del.

TUBIFEX.

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